ACUTE HYPOGLYCAEMIA IN MAN

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"It is the same in the case of numberless pathological lesions which are real experiments, by which physicians and physiologists profit, without any purpose on their part to produce the lesions, which result from disease. I emphasise this idea now, because it will be useful to us later, to prove that medicine includes real experiments which are spontaneous, and not produced by physicians".

Claude Bernard (1813 - 1878)

ABSTRACT OF THESIS (Regulation 6.9)

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Title of Thesis ACUTE HYPOGLYCAEMIA IN MAN

Insulin-induced hypoglycaemia in man provokes an integrated hormonal and metabolic response to restore normoglycaemia. Secretion of counterregulatory hormones promote hepatic glycogenolysis and gluconeogenesis, and mobilise substrates from extra-hepatic tissues. Both adrenergic and cholinergic neural mechanisms are activated by hypoglycaemia, but their contributions to the control of metabolic recovery are not clear. Activation of the sympatho-adrenal system does not appear to be essential for blood glucose recovery.

The effect of acute hypoglycaemia on pancreatic beta cell function in response to a subsequent meal was studied in normal subjects. Suppression of endogenous insulin secretion following hypoglycaemia persisted until ingestion of a meal at 210 minutes after insulin injection. After the meal carbohydrate intolerance was associated with an abnormal pattern of insulin secretion, characterised by a delay in the early post-prandial rise of plasma insulin and late hyperinsulinaemia. Investigations of the possible underlying mechanisms implicated glucopenia of the pancreatic beta cells.

The contributions of adrenergic and cholinergic mechanisms to metabolic recovery from hypoglycaemia were studied in tetraplegic subjects with a pre-ganglionic sympathectomy (adrenergic denervation), one group of whom were given atropine (combined adrenergic denervation and cholinergic blockade). Blood glucose recovery was impaired only in the tetraplegic subjects given atropine. Both tetraplegic groups demonstrated a reduced blood lactate response, a delayed rise of plasma free fatty acids and an absent cyclic AMP response. Changes in pancreatic glucagon and C-peptide following hypoglycaemia were appropriate to blood glucose values, and were not influenced significantly by islet denervation. Stimulation of glucagon secretion occurred independent of cholinergic vagal control. In the tetraplegic subjects given atropine, the plasma cortisol response was impaired and the pattern of ACTH secretion was abnormal, suggesting possible blockade of central cholinergic receptors at hypothalamic level. This cortisol deficiency may explain the delayed blood glucose recovery in these subjects.

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CONTENTS

SUMMARY

Chapter 1 Metabolic and hormonal responses to acute hypoglycaemia in man

The first clinical descriptions of hypoglycaemia followed the introduction of insulin for the treatment of diabetes mellitus, and early investigators demonstrated that activation of the sympatho-adrenal system was involved in the metabolic response. The restoration of normoglycaemia following insulin-induced hypoglycaemia results from the production of glucose by hepatic glycogenolysis and gluconeogenesis, while substrates for gluconeogenesis are released from extrahepatic tissues. Glycogenolysis in muscle liberates lactate, lipolysis in adipose tissue releases free fatty acids (FFA) and glycerol, and glucogenic amino acids are released from muscle, of which alanine is the most important. The principal metabolic pathways in the production of glucose are described.

The important counterregulatory hormones which are secreted in response to hypoglycaemia are adrenaline, noradrenaline, pancreatic glucagon, growth hormone and cortisol. Catecholamines promote hepatic and muscle glycogenolysis and lipolysis in adipose tissue, mainly via beta-adrenergic mechanisms. Glucagon acts by stimulating hepatic glycogenolysis and gluconeogenesis. The actions of cortisol and growth hormone on metabolic processes following hypoglycaemia are not defined clearly, but these hormones may exert a permissive action on the effects of other counterregulatory hormones.

The release of counterregulatory hormones leads to the production of glucose stored as hepatic glycogen, and the mobilisation of substrates for gluconeogenesis, as an integrated metabolic response to hypoglycaemia to maintain the supply of glucose which is essential for cerebral function.

SUMMARY

Chapter 2 Neural control of the recovery from acute hypoglycaemia

The anatomical organisation and chemical subdivisions of the autonomic nervous system are described briefly with reference to the involvement of adrenergic and cholinergic mechanisms in the metabolic recovery from hypoglycaemia. Sympathetic and parasympathetic centres which respond to hypoglycaemia are located within the hypothalamus. The two principal methods for inducing a glucopenic stress are the administration of intravenous insulin and the infusion of 2-deoxy-D-glucose, a competitive inhibitor of glucose.

The methods of investigating adrenergic mechanisms in the response to hypoglycaemia include the measurement of plasma and urinary catecholamines, pharmacological blockade of adrenergic receptors, and the effect of hypoglycaemia on either adrenalectomised or sympathectomised subjects. These studies show that circulating catecholamines promote the haemodynamic changes which occur in response to hypoglycaemia, and adrenergic mechanisms mediate the mobilisation of FFA and lactate. However activation of the sympatho-adrenal system alone does not appear to be essential for blood glucose recovery from hypoglycaemia.

Cholinergic mechanisms have been studied using pharmacological blockade with atropine and ablation studies which produce parasympathetic denervation (vagotomy). The cholinergic manifestations of hypoglycaemia are mediated via the parasympathetic and sympathetic divisions and include haemodynamic changes, salivation, sweating, vasodilatation and the secretion of certain gastrointestinal and pancreatic hormones, including pancreatic polypeptide. In particular, evidence is presented which suggests that the secretion of pancreatic glucagon is influenced by vagal (cholinergic) innervation.

SUMMARY

Chapter 3 Rationale and aims of experimental investigations of hypoglycaemia in man

This chapter describes the rationale for the experimental studies described in Chapters 4 - 8. These studies are concerned with two areas related to metabolic recovery from acute hypoglycaemia in man. A brief introduction to each area of investigation is provided and the aims of the studies are outlined.

(1) The effect of acute hypoglycaemia on pancreatic beta cell function in response to a subsequent meal: the development of an assay for plasma C-peptide has provided an index of endogenous insulin secretion during insulin-induced hypoglycaemia. This has demonstrated prolonged suppression of pancreatic beta cell secretion following hypoglycaemia in man. Previous studies have suggested that glucose intolerance may occur after hypoglycaemia at varying times after insulin administration. The effect of hypoglycaemia on pancreatic beta cell function in man in response to a subsequent meal has not been examined previously.

(2) Neural control of pancreatic islet cell function and the metabolic and hormonal responses to hypoglycaemia: tetraplegic subjects, who have a pre-ganglionic sympathectomy,provide a human model for studying the effect of adrenergic denervation on pancreatic islet cell function in response to hypoglycaemia. The addition of atropine to these subjects to produce cholinergic blockade enables an assessment of cholinergic mechanisms, and no human studies have examined previously the effect of combined sympathetic and parasympathetic blockade on recovery mechanisms from insulin-induced hypoglycaemia. The relative contribution of the principal counterregulatory hormones to metabolic recovery from hypoglycaemia can also be examined.

SUMMARY

Chapter 4 The effect of acute hypoglycaemia on the pancreatic beta cell response to a meal in man

The plasma concentrations of C-peptide, insulin and glucose were measured in nine healthy subjects during insulin-induced hypoglycaemia followed by a meal. Identical observations were made in the same subjects after an equivalent period of fasting without hypoglycaemia (control study). Endogenous secretion of insulin was suppressed following administration of exogenous insulin, and this persisted long after the blood glucose concentration had returned to normal. After the meal blood glucose rose to a peak at 60 min and was still raised at 120 min, compared to the small transient rise of blood glucose observed in the control study. This carbohydrate intolerance was associated with an abnormal pattern of insulin secretion, characterised by a delay in the early post-prandial rise of plasma insulin, and a pronounced elevation two hours after the meal.

Acute hypoglycaemia may thus induce an abnormal pattern of insulin secretion in response to a subsequent meal, with the development of impaired carbohydrate tolerance in normal subjects.

SUMMARY

Chapter 5 The mechanism of the abnormal pancreatic beta cell response to food following acute hypoglycaemia in man

Following acute insulin-induced hypoglycaemia, an abnormal pattern of insulin secretion in response to a meal was confirmed in six normal subjects. This was characterised by an initial impairment of insulin secretion and late hyperinsulinaemia. Post-prandial gastrointestinal hormone levels were normal following hypoglycaemia in these subjects. In four other subjects, the administration of intravenous glucose prior to the meal partially reversed the abnormal pattern of insulin secretion. In two normal subjects, aminophylline infusion before the meal did not improve the abnormal secretory response of insulin. In two subjects with a total pre-ganglionic sympathectomy, the patterns of response of blood glucose, plasma insulin and C-peptide were similar to those observed following hypoglycaemia in normal subjects. It is unlikely therefore that either an abnormal entero-insular axis, an elevation of plasma catecholamine concentrations or depletion of cyclic AMP within the pancreatic beta cells have a primary role in the production of this phenomenon. This effect of hypoglycaemia on post-prandial insulin secretion may be caused, at least in part, by glucopenia of the pancreatic beta cells.

SUMMARY

Chapter 6 Manifestations of acute hypoglycaemia in subjects with pre-ganglionic sympathectomy with and without cholinergic blockade

The effects of acute insulin-induced hypoglycaemia were observed on 11 normal subjects, six tetraplegic subjects and six tetraplegic subjects given atropine. The tetraplegic subjects without atropine experienced mild neuroglycopenia only, whereas tetraplegic subjects given atropine experienced severe and prolonged neuroglycopenia. The normal haemodynamic changes and sweating were absent in the tetraplegic subjects, but a small gradual increase in pulse rate was noted following hypoglycaemia. Systolic and diastolic blood pressures were subnormal. The adequacy of cholinergic blockade in the tetraplegic subjects given atropine was confirmed by a sustained elevation in pulse rate. The normal rise in haematocrit in response to hypoglycaemia was absent in the tetraplegic subjects, and mean basal plasma noradrenaline was lower than in normal subjects with no measurable response to hypoglycaemia.

The haemodynamic changes in normal subjects were mediated by activation of the sympatho-adrenal system. In tetraplegic subjects the increase in heart rate may be caused by a withdrawal of vagal cardiac tone in response to the fall in blood pressure, resulting from the inability to initiate activation of the sympathetic nervous system in response to hypoglycaemia. In normal subjects the sweating response to hypoglycaemia appears to be mediated by a sympathetic cholinergic mechanism and not by circulating catecholamines.

SUMMARY

Chapter 7 Plasma substrate responses to acute hypoglycaemia in man: adrenergic and cholinergic mechanisms

The contributions of adrenergic and cholinergic mechanisms to the metabolic recovery from acute hypoglycaemia induced by insulin wereexamined in 11 normal subjects, six tetraplegic subjects (adrenergic denervation) and six tetraplegic subjects given atropine (combined adrenergic denervation and cholinergic blockade).

Blood glucose recovery was impaired only in the tetraplegic subjects given atropine. The blood lactate response was reduced and the rise of plasma free fatty acids was delayed in both groups of tetraplegic subjects, in whom the normal rise of plasma cyclic AMP was also absent.

Blood glucose homeostasis was impaired therefore during combined adrenergic denervation and cholinergic blockade. The impaired blood lactate and plasma free fatty acid responses in the tetraplegic subjects indicate the role of adrenergic mechanisms in the activation of muscle glycogenolysis and adipose tissue lipolysis. The absent response of plasma cyclic AMP implicates mediation by catecholamines in the activation of hepatic adenyl cyclase in response to hypoglycaemia.

SUMMARY

Chapter 8 Hormonal response to acute hypoglycaemia in man: neural control of pancreatic islet cell function and hormonal mediation of metabolic homeostasis

The role of autonomic neuro-regulation of pancreatic islets in response to hypoglycaemia was examined in 11 normal subjects, six tetraplegic subjects (adrenergic denervation) and six tetraplegic subjects given atropine (combined adrenergic denervation and cholinergic blockade). Changes in plasma pancreatic glucagon and C-peptide following hypoglycaemia were appropriate to blood glucose values and were not influenced significantly by islet denervation. Thus, neuro-regulation of human pancreatic islet function in response to hypoglycaemia may be of limited importance, and stimulation of pancreatic glucagon secretion apparently can occur in the absence of cholinergic vagal control.

Growth hormone levels were higher in both tetraplegic groups in response to hypoglycaemia. The normal rise in plasma pancreatic polypeptide was absent in the tetraplegic subjects given atropine, which confirms the role of vagal cholinergic control of secretion of this hormone. The plasma cortisol response was impaired in the tetraplegic subjects given atropine despite a greater degree of hypoglycaemia, and in these subjects the pattern of ACTH secretion was abnormal. A blockade of central cholinergic receptors producing impaired activation of ACTH secretion at hypothalamic level may explain, at least in part, the delayed restoration of normoglycaemia in the tetraplegic subjects given atropine.

PART I THEORETICAL BACKGROUND

CHAPTER 1

METABOLIC AND HORMONAL RESPONSES TO ACUTE HYPOGLYCAEMIA IN MAN

CHAPTER 1

METABOLIC AND HORMONAL RESPONSES TO ACUTE HYPOGLYCAEMIA IN MAN

1. Historical perspective

2. The recovery from acute hypoglycaemia

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      Gluconeogenesis
      Lipolysis
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   B. Counterregulatory hormonal response to hypoglycaemia

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      Catecholamines
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      Growth hormone

   D. The integrated hormonal and metabolic response for blood glucose recovery from hypoglycaemia

1. HISTORICAL PERSPECTIVE

The biblical account of Esau is thought to be the first recorded description of symptomatic hypoglycaemia in man (Genesis, Ch. 25, v. 29 - 34). Despite prolific investigation of carbohydrate metabolism during the nineteenth century by Claude Bernard and others, the lack of accurate methods to measure blood glucose prevented an exposition of acute hypoglycaemia in man until the impetus provided by the introduction of insulin therapy in 1922. Hypoglycaemia had been recognised earlier as a biochemical anomaly associated with phosphorus poisoning and the administration of drugs such as hydrazine. The mildly hypoglycaemic effect of salicylates was described by Ebstein in 1876, and in 1916 Fischler and his collaborators described symptoms of excitement, convulsions, collapse and coma which accompanied the low blood sugar produced by phlorizin. They described this condition as "glycoprival intoxication".

The development of the concept of hormones by Bayliss and Starling (1902 - 1906) stimulated further attempts to extract insulin from the pancreas. Georg Zuelzer prepared pancreatic extracts and administered them to dogs between 1903 and 1914, but the "toxic" phenomena which occurred included loss of consciousness and convulsions, which with hindsight were probably caused by hypoglycaemia. His experiments, although successful, tragically were misinterpeted and had to be abandoned at the outbreak of the First World War. A similar fate overtook the Roumanian, Paulesco, who in 1916 had an effective though less purified pancreatic extract at his disposal. Publication of his findings was delayed for years by the First World War, and was eclipsed by work from Canada. In the summer of 1921, the Canadian orthopaedic surgeon, Frederick Banting, and a

medical student Charles Best, demonstrated that the injection of a crude pancreatic extract lowered the blood glucose of pancreatectomised dogs. They observed that insulin could not only lower the blood glucose of normal and diabetic animals, but that it could also produce signs of cerebral dysfunction, including convulsions and coma, that could be reversed by restoring blood glucose to normal (Banting et al., 1922).

The therapeutic application of insulin for the treatment of human diabetes provoked the first detailed descriptions of the symptoms and signs of acute hypoglycaemia in man (Fletcher and Campbell, 1922; Banting et al., 1923). Profuse sweating, dilatation of the pupils, skin pallor and an increase in pulse rate were noted to accompany subjective sensations of anxiety, emotional instability or excitement, coldness and faintness. As the level of blood glucose decreased further, signs of dysarthria, disorientation and confusion preceded the eventual loss of consciousness. The treatment recommended by these authors was the administration of food or orange juice, and the injection of "epinephrin" if the patient was unconscious.

The clinical signs of hypoglycaemia implied an increase in activity of the sympathetic nervous system, and in 1924 two groups of investigators described independently the stimulatory effect of hypoglycaemia on adrenal medullary secretion. The Boston physiologist Walter Cannon and his associates observed that insulin-induced hypoglycaemia produced a release of "adrenin" into the circulation of unanaesthetised cats (Cannon et al., 1924). The effect on the denervated heart was used as an index of circulating hormone from the adrenal medulla. The removal of one adrenal gland with concurrent denervation of the other prevented release of this hormone, as did correction of the blood glucose with dextrose. Houssay et al., (1924) used cross-circulation experiments in dogs to show a similar secretion

from the adrenal medulla in response to hypoglycaemia. This endocrine response could also be prevented by sympathetic denervation of the adrenal glands or the intravenous injection of glucose. These early classical experiments of the endocrine response to hypoglycaemia indicated a role for the sympatho-adrenal system in the metabolic recovery from hypoglycaemia, and this was confirmed by further studies on adrenalectomised and sympathectomised animals (Britton et al., 1928; Dworkin, 1931; Schlossberg et al., 1933; Freeman et al., 1934). The practical difficulties of developing reliable assays for adrenaline and noradrenaline hindered further progress in this area for several decades. Von Euler and Luft (1952) eventually showed that the urinary excretion of catecholamines was increased following hypoglycaemia in man.

The hyperglycaemic effect of adrenaline was observed in animals at the beginning of this century (Blum, 1901) and subsequent investigators were aware that activation of the sympatho-adrenal system in response to hypoglycaemia promoted release of glucose by the liver. Cannon et al., (1924) stated that "both the nerve impulses (sympathetic) and the secreted adrenin have the effect of liberating sugar from the liver into the circulation, thus tending to restore the disturbed equilibrium. This may be regarded as a first line of defense against a falling glycemic concentration". The central role of the liver in the preservation of glucose homeostasis had already been postulated by Claude Bernard in 1876 with his concept of maintenance of the "milieu intérieur". He had earlier described glycogen as a temporary storage form of carbohydrate and had discovered the ability of the liver to maintain blood glucose levels in the absence of carbohydrate in the diet, thus invoking the concept of carbohydrate homeostasis (Bernard, 1853). This was demonstrated

conclusively by the production of severe hypoglycaemia following total hepatectomy in dogs with its consequent adverse effect on cerebral function (Mann and Magath, 1921; 1922). These early observations were refined by Soskin et al., (1938) who showed that the perfused dog liver removed glucose from portal venous blood when the glucose concentration was high, and conversely the perfused liver produced glucose when the portal blood glucose was low.

Although glucagon had been identified as a potential glucoregulatory hormone in 1923 (Kimball and Murlin, 1923), the role of glucagon and other counterregulatory hormones in the metabolic response to hypoglycaemia had to await the development of modern radioimmunoassay techniques in the early 1960's.

Finally the participation of neural factors in the metabolic recovery from hypoglycaemia had been suspected since the original observation by Claude Bernard in 1849 that hyperglycaemia develops in experimental animals after puncture of the floor of the fourth ventricle. Paul Langerhans described an innervation of the pancreatic islets in his doctoral thesis in 1869, but research into neural regulation of pancreatic islet function was discouraged by the failure of early studies to show that denervation of the pancreas had any effect on the regulation of blood glucose (Allen, 1922). Subsequent research in the modern era has shown however that pancreatic endocrine function can be modulated by neural influences, although the contribution of the autonomic nervous system to the control of pancreatic endocrine responses to hypoglycaemia is far from clear. The pancreatic islets are exposed to a complex array of metabolic, endocrine and neural influences which control normal pancreatic endocrine secretion, and presumably modify the responses to abnormal metabolic states such as hypoglycaemia.

2. THE RECOVERY FROM ACUTE HYPOGLYCAEMIA

Under normal conditions the predominant metabolic fuel used by the central nervous system is glucose. The brain metabolises 6G of glucose per hour (Reinmuth et al., 1965), and its daily requirement has been estimated to be 110 - 145G, (Owen et al., 1967). A rapid fall in blood glucose affects the central nervous system in a similar way to a decrease in arterial oxygen, producing immediate effects on neuronal activity. Prolonged hypoglycaemia produces irreversible structural damage, causing loss of brain stem function and death. Because of the vital importance of glucose for cerebral function, several homeostatic mechanisms exist to maintain normoglycaemia, and severe hypoglycaemia therefore represents an unphysiological state that does not normally occur during life.

The normal range of blood glucose after an overnight fast usually varies from 3.0 - 5.5 mmol/l (approximately 55 - 100mg/100ml), and traditionally in clinical practice hypoglycaemia has been considered to occur when the blood glucose falls below 2.5 mmol/l. The physiological response to acute hypoglycaemia is triggered by a fall in blood glucose below 2.9 mmol/l in normal human subjects (DeFronzo et al., 1974; Young et al., 1974), although recent studies suggest that there is no absolute threshold of blood glucose required to activate the counterregulatory systems (Cryer, 1980). The magnitude of the counterregulatory response appears to be inversely related to the absolute concentration of blood glucose, so that a decrease of blood glucose to a hypoglycaemic level releases more counterregulatory hormones than a decrease of blood glucose from a high level to one within the physiological range (Santiago et al., 1980).

A. METABOLIC PROCESSES FOR RESTORING NORMOGLYCAEMIA

The prime necessity in the metabolic response to hypoglycaemia is the immediate provision of glucose. This is released rapidly from the liver following the activation of hepatic glycogenolysis and gluconeogenesis. In extrahepatic tissues, glycogenolysis in skeletal muscle liberates lactate, and lipolysis in adipose tissue provides free fatty acids (FFA). Protein hydrolysis releases glucogenic amino acids from muscle, which provide substrate for hepatic gluconeogenesis. The principal glucogenic amino acid is alanine. The main pathways of carbohydrate utilisation and glucose production are shown in Fig. 1.1. The mechanisms which initiate and regulate these processes are incompletely understood, and will be discussed in Chapter 2.

Glycogenolysis: The control of hepatic glycogenolysis has recently been reviewed comprehensively (Hems and Whitton, 1980). The degradation of glycogen is catalysed by glycogen phosphorylases. The product is glucose-1-phosphate; the glucose carbon skeleton derived from liver glycogen can therefore follow the pathways of the hexose phosphates (Fig. 1.1) to free glucose, end products of glycolysis and products of acetyl Coenzyme A (CoA) metabolism. The main product of hepatic glycogenolysis in response to hypoglycaemia is free glucose which is released rapidly into the blood.

In skeletal muscle, activation of phosphorylase promotes glycogenolysis to glucose-6-phosphate, but glucose is not formed in the absence of a muscle glucose-6-phosphatase. The glycolytic and oxidative breakdown of the glucose-6-phosphate produces lactate which diffuses into the blood and provides a significant precursor for hepatic gluconeogenesis. Reconversion of lactate to pyruvate,

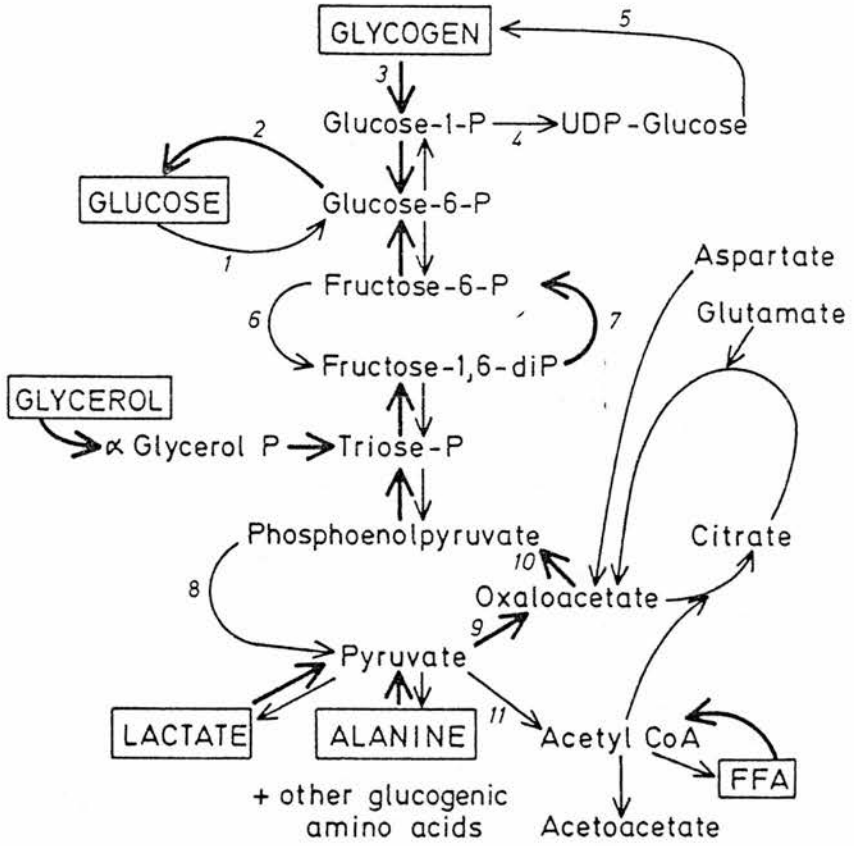

Fig. 1.1 Main metabolic pathways in the liver involved in glucose production in response to hypoglycaemia, with the direction of response indicated by the thicker arrows. The enzymes which regulate the principal steps in glucose synthesis or glycolysis are numbered as shown:

GLYCOLYTIC ENZYMES		SYNTHETIC ENZYMES	
1	hexokinase, glucokinase	2	glucose-6-phosphatase
3	phosphorylases	4	UDPG pyrophosphorylase
6	phosphofructokinase	5	glycogen synthetase
8	pyruvate kinase	7	fructose diphosphatase
11	pyruvate dehydrogenase	9	pyruvate carboxylase
		10	PEP carboxykinase

glucose-6-phosphate and glucose occurs in the liver (the "Cori cycle") and in starvation may account for 20 - 40 per cent of hepatic glucose output (Kreisberg et al., 1970).

Gluconeogenesis: The liver is capable of producing glucose from various substrates including lactate, pyruvate, glycerol, the glucogenic amino acids and odd-chain fatty acids. All these substances are glucogenic, and their conversion to glucose requires several key enzyme systems which enable the liver to produce new glucose-6-phosphate from non-carbohydrate sources. The main synthetic enzymes involved in glycolysis and gluconeogenesis in the liver are shown in Fig. 1.1. Regulation of these processes occurs at certain stages of the pathway, and usually at sites where two or more enzymes are involved. Because of the enzyme pyruvate carboxylase, pyruvate can be converted directly into oxaloacetate, and phosphoenolpyruvate (PEP) carboxylase catalyses the formation of PEP from oxaloacetate. Substances which can be transformed to oxaloacetate can therefore be used for gluconeogenesis; lactate and alanine thereby can enter the gluconeogenic pathway. The activity of pyruvate carboxylase is regulated by the concentration of acetyl-CoA (Scrutton and Utter, 1968), which is abundant when fatty acid metabolism is enhanced. This is one of several regulatory devices which favour gluconeogenesis.

Lipolysis: Hypoglycaemia induces the activation of lipase in adipose tissue, thus stimulating the lipolysis of triglycerides and the release of free fatty acids and glycerol. Glycerol can enter the gluconeogenic pathway via conversion to alpha-glycerolphosphate, but is thought to be a relatively minor gluconeogenic substrate (Cahill, 1970). Free fatty acids are oxidised in the liver to two-carbon fragments and acetyl-CoA (Fig. 1.1) and provide substrate for glucose synthesis.

Glucogenic amino acids: A significant proportion of newly-formed glucose originates from glucogenic amino acids derived from protein hydrolysis. Studies in prolonged starvation have indicated that alanine is quantitatively the most important three-carbon precursor (other than lactate) for hepatic gluconeogenesis (Felig et al., 1969; Owen et al., 1969). Data from the infusion of labelled substrates in man after an overnight fast indicate that alanine (Garber et al., 1976) and lactate (Kreisberg et al., 1970) each contribute approximately one-seventh of the total glucose production.

The contribution of alanine to glucose production in response to hypoglycaemia has been described in detail by Garber et al., (1976) using isotope dilution techniques to measure the simultaneous turnover of glucose and alanine, and their precursor-product interrelationships. In the steady state prior to insulin administration the amount of glucose derived from alanine was only 13 per cent, which is consistent with measurements using arterio-hepatic venous catheterisation techniques in fasting man (Felig et al., 1970). Total glucose production and gluconeogenesis from alanine was inhibited markedly after insulin injection. Although gluconeogenesis from alanine returned to basal values within 30 minutes of insulin administration, it did not rise above basal until 60 minutes after insulin. However by this time total glucose production had doubled, and it seems likely that the initial increase in total glucose production resulted principally from accelerated glycogenolysis and not from gluconeogenesis. The contribution of alanine to gluconeogenesis increased steadily in the second hour after insulin administration, but did not derive entirely from an increased rate of alanine delivery to the liver, since the alanine inflow was increased by only 15 - 20

per cent. The increased rate of gluconeogenesis appeared therefore to result from a redirection of intrahepatic alanine metabolism.

B. COUNTERREGULATORY HORMONAL RESPONSE TO HYPOGLYCAEMIA

Acute hypoglycaemia in man is characterised by diverse changes in plasma substrate and hormone concentrations which are accompanied by simultaneous haemodynamic responses. Several hormones are secreted in response to acute hypoglycaemia but few of these have metabolic actions which can potentially reverse hypoglycaemia. The hormones which are secreted in response to hypoglycaemia and are considered also to have significant counterregulatory effects include the catecholamines, adrenaline and noradrenaline (Vendsalu, 1960; Christensen et al., 1975), pancreatic glucagon (Gerich et al., 1974), growth hormone (Roth et al., 1963) and cortisol (Landon et al., 1963; Greenwood et al., 1966).

The temporal sequence of release of these hormones in response to acute hypoglycaemia has been examined in man (Gerich et al., 1974; Garber et al., 1976). After the intravenous injection of insulin (0.15 units/kg body weight), a significant increase of plasma adrenaline is observed at 20 minutes and plasma noradrenaline at 25 minutes, with maximal levels of both catecholamines occurring at 50 minutes (Garber et al., 1976). Plasma glucagon increases significantly at 30 minutes with a maximal response at 40 minutes, plasma cortisol rises at 30 minutes with a maximal response at 60 minutes, but growth hormone increases relatively slowly after insulin, with a significant increment at 40 minutes and a peak concentration at 60 minutes. Gerich et al., (1974) demonstrated that the peak response of glucagon precedes those of cortisol

and growth hormone by 15 to 30 minutes. In contrast, a significant increase in plasma glucagon has been reported prior to the rise in plasma catecholamine levels and the onset of glucose counterregulation by Rizza et al., (1979a), but in this study a much smaller dose of insulin (0.04 units/kg) was used to induce hypoglycaemia. The temporal sequence of hormonal secretion in relation to the compensatory increase in glucose production suggests a role for all of these hormones in maintaining glucose homeostasis, but the individual contribution of each hormone has not been clearly defined.

C. EFFECTS OF COUNTERREGULATORY HORMONES ON METABOLIC PATHWAYS AND SUBSTRATE AVAILABILITY

Insulin produces hypoglycaemia by stimulating the hepatic and peripheral uptake of glucose, and by profoundly inhibiting total glucose production (Garber et al., 1976). Insulin inhibits protein breakdown and decreases net amino acid release (Pozefsky et al., 1969; Blackshear et al., 1974) which inhibits the inflow of glucogenic amino acids into the blood. It also inhibits glycerol and lactate release in functionally hepatectomised rats (Blackshear et al., 1974) and has a marked antilipolytic action. This has been demonstrated *in vitro* in adipose tissue and is due at least in part to a reduction in cyclic AMP (cAMP) levels with a consequent decrease in activity of cAMP-dependent protein kinase and hormone-sensitive lipase (Soderling et al., 1973). Infusion of physiological amounts of insulin into the forearms of fasting human subjects inhibits the net release of free fatty acids, presumably from adipose tissue (Pozefsky et al., 1969).

The counterregulatory hormones antagonise the actions of insulin and act to increase glucose production and decrease glucose utilisation.

Catecholamines: Adrenaline and noradrenaline promote rapid glycogenolysis in the liver to provide glucose, glycogenolysis in muscle to liberate lactate, and lipolysis in adipose tissue to provide FFA (Young and Landsberg, 1977). Substrates thereby are provided for hepatic gluconeogenesis which is enhanced by catecholamine-induced effects at several stages of the gluconeogenic pathway. Catecholamines are potent lipolytic agents and also promote ketogenesis. While ketogenesis is usually attributed to the stimulation of lipolysis, catecholamines may stimulate ketogenesis through direct or indirect (hormone-mediated) effects on hepatic metabolism (Schade and Eaton, 1979).

Catecholamines appear to exert their effects in the liver via interaction with adrenergic receptors, and not via mediation by cAMP. In vitro studies of rat liver have demonstrated that beta-adrenergic blockade with propranolol inhibits the increase in cAMP induced by adrenaline, without preventing the increase in phosphorylase activation and hepatic glycogenolysis (Sherline et al., 1972). Tolbert et al., (1973) showed that propranolol, while blocking cAMP accumulation, did not prevent the stimulation of gluconeogenesis by adrenaline in isolated liver cells. They also showed that the beta agonist, isoproterenol, raised cAMP concentrations without stimulating gluconeogenesis, which was however stimulated by the alpha agonist, phenylephrine. It was concluded that the alpha-receptor effects of adrenaline include an increase in phosphorylase activity with an associated decrease in glycogen synthetase activity, thus leading to hepatic glycogenolysis (Hutson et al., 1976; Cherrington and Exton, 1976). Glucose uptake by muscle is decreased (Groen et al., 1966; Abramson and Arky, 1968) and glucose clearance from the circulation is reduced by catecholamines (Rizza et al., 1979b).

Catecholamines also enhance glycerol and lactate output from peripheral tissues by interaction with beta-adrenergic receptors in adipose tissue and muscle (Exton, 1979). This interaction is thought to activate adenyl cyclase and cAMP-dependent enzymes which promote glycolysis, lactate production and lipolysis.

Recent evidence has indicated that the direct effects of adrenaline on glucose delivery and clearance in man are mediated mainly by beta-adrenergic mechanisms (Rizza et al., 1979b; Deibert and DeFronzo, 1979; Rizza et al., 1980) while indirect effects resulting from the inhibited secretion of insulin are mediated by alpha-adrenergic mechanisms.

Glucagon: Glucagon exerts potent effects on hepatic metabolism but has minimal effects on peripheral tissues. It converts hepatic glycogen phosphorylase from its inactive to its active form to promote glycogenolysis (Parilla et al., 1974), but has no effect on muscle phosphorylase. It rapidly activates gluconeogenesis from lactate, pyruvate, alanine and glycerol in the perfused rat liver and in isolated rat hepatocytes and rapidly inhibits hepatic glycolysis (Exton, 1979). These effects can be mimicked by exogenous cAMP and are preceded by a rise in this nucleotide with activation of cAMP-dependent protein kinase (Cherrington and Exton, 1976). The actions of glucagon are therefore thought to be mediated via cAMP. However, efforts to link cAMP or protein kinase to the phosphorylation or activation of specific gluconeogenic enzymes have not been successful, and the precise sites of action of glucagon in this process remain undetermined.

Glucagon is thought to influence the redirection of hepatic alanine metabolism during gluconeogenesis (Chiasson et al., 1975), since glucagon has been shown to stimulate hepatic gluconeogenesis

from alanine without increasing alanine extraction by the liver. The effect of a physiological deficiency of glucagon has been shown in the dog by suppression of endogenous glucagon by somatostatin infusion (Jennings et al., 1977). The conversion of ^{14}C-alanine to ^{14}C-glucose was decreased under these conditions, but gluconeogenesis returned to normal when physiological levels of plasma glucagon were restored. Finally, glucagon increases intrahepatic lipolysis, probably via cAMP activation (Bewsher and Ashmore, 1966).

Cortisol: The contribution of cortisol to blood glucose recovery from hypoglycaemia is not well defined. It stimulates the release of amino acids from extrahepatic tissues, thus providing increased substrate for gluconeogenesis, while decreasing the sensitivity of peripheral tissues to insulin and reducing the peripheral uptake of glucose (Baxter and Forsham, 1972). In adipose tissue cortisol promotes lipolysis, augmenting the effect of growth hormone in vitro (Fain, 1967). In addition, cortisol exerts permissive effects on the actions of glucagon and catecholamines on hepatic glycogenolysis and gluconeogenesis (Exton et al., 1972). Gluconeogenesis is influenced by the effects of cortisol on the catecholamine-mediated release of lactate from skeletal muscle and glycerol from adipose tissue (Steele, 1975). A gluconeogenic response to adrenaline or glucagon was not observed in the livers of fasted adrenalectomised rats, and neither gluconeogenesis nor glycogenolysis in livers from fed steroid-deficient rats was stimulated by low levels of these hormones (Exton et al., 1972). Cortisol deficiency thus appears to impair the sensitivity of gluconeogenesis and glycogenolysis to the stimulatory action of adrenaline and glucagon.

Growth hormone: Although growth hormone has not been shown convincingly to alter gluconeogenesis or glycogenolysis directly, it does appear

to antagonise some actions of insulin, and it decreases peripheral glucose utilisation (Fineberg and Merimee, 1974). Growth hormone increases fatty acid and glycerol release in vivo and in vitro by enhancing triglyceride breakdown and depressing lipogenesis (Fain, 1967; Gerich et al., 1976), but this effect is slow and apparently involves protein synthesis. Studies of hypophysectomised rats suggest that in the absence of growth hormone, amino acid utilisation for gluconeogenesis in the liver is enhanced, which would indicate a long-term inhibitory action of growth hormone on hepatic gluconeogenesis (Tolman et al., 1973). Although glucose production is not increased acutely by growth hormone or cortisol (Exton et al., 1970), both hormones may assist glucose production by inducing hepatic resistance to insulin through interference with insulin-receptor binding (Kahn et al., 1978). The overall importance of growth hormone to blood glucose recovery from hypoglycaemia is debatable, and recently its role has been questioned (Feldman et al., 1975; Rizza et al., 1979a).

D. THE INTEGRATED HORMONAL AND METABOLIC RESPONSE FOR BLOOD GLUCOSE RECOVERY FROM HYPOGLYCAEMIA

The numerous factors which stimulate and regulate the metabolic recovery from hypoglycaemia operate simultaneously and interact in a variety of ways in the intact organism. Having outlined the metabolic pathways and the actions of the individual hormones which are involved in the metabolic recovery from hypoglycaemia, the integrated response can now be reviewed. The principal metabolic events which occur following hypoglycaemia are presented in Fig. 1.2.

After the initial effects of exogenous insulin to induce acute hypoglycaemia, a compensatory increase in glucose production occurs

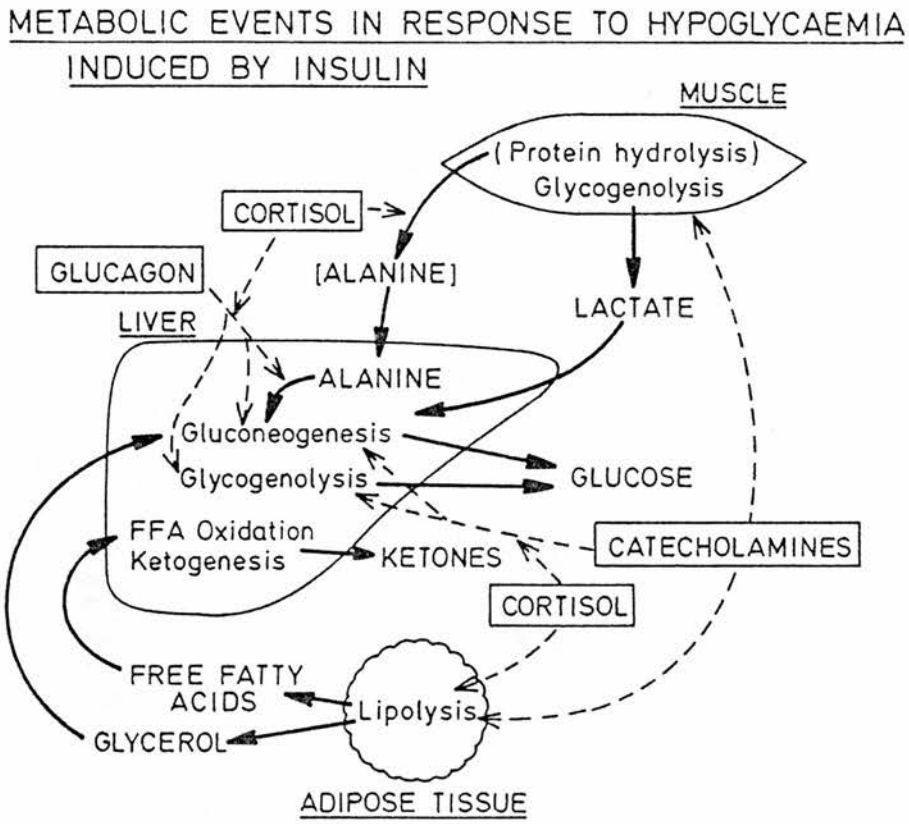

Fig. 1.2 The main metabolic events which occur in response to hypoglycaemia induced by insulin. The effects of the principal counterregulatory hormones on metabolic processes are indicated (dotted lines).

rapidly in the liver to restore normoglycaemia, despite the persisting presence of significant levels of plasma insulin at that time (Garber et al., 1976). Hepatic glucose output rises while glucose uptake in peripheral tissues is decreased. The dramatic early increase in hepatic glucose output appears primarily to reflect glycogenolysis in the liver. This increase in hepatic glucose production by the stimulation of hepatic glycogenolysis and gluconeogenesis is activated by catecholamines and glucagon, with cortisol exerting a permissive influence. Catecholamines, by their effects on lipolysis in adipose tissue and glycogenolysis in skeletal muscle, also stimulate the provision of substrates suitable for gluconeogenesis. This is demonstrated by the marked increase in circulating lactate, free fatty acids and glycerol.

Although plasma alanine levels do not change significantly following hypoglycaemia, and only minor changes in alanine flux occur between the liver and the blood, gluconeogenesis from alanine rises gradually and continually to suprabasal levels, between one and two hours after insulin administration (Garber et al., 1976). Although growth hormone and cortisol may contribute to the increase in glucose production by a more permissive role such as the induction of hepatic resistance to insulin binding, the alteration of substrate availability or the promotion of enzyme induction, neither hormone increases glucose production acutely (Exton et al., 1970).

There is evidence to indicate that endogenous insulin secretion is suppressed during the recovery from hypoglycaemia (Sando et al., 1970; Turner et al., 1973b; Horwitz et al., 1975b), and this may represent a further homeostatic mechanism to encourage normal blood glucose recovery. The effect of hypoglycaemia on endogenous insulin secretion will be discussed further in chapters 3 and 4.

CHAPTER 2

NEURAL CONTROL OF THE RECOVERY FROM ACUTE HYPOGLYCAEMIA

Chapter 2

Neural control of the recovery from acute hypoglycaemia

1. Autonomic nervous system

   A. Anatomical organisation

      (a) Parasympathetic division
      (b) Sympathetic division

   B. Chemical subdivisions

      (a) Cholinergic neurons
      (b) Adrenergic neurons

   C. Cholinergic and adrenergic receptors

   D. Hypothalamic control centres in hypoglycaemia

2. Methods of inducing glucopenia

      (a) Insulin
      (b) 2-deoxy-D-glucose

3. Adrenergic mechanisms in the response to hypoglycaemia

      (a) Methods of investigation
      (b) Secretion of catecholamines
      (c) Effect of pharmacological blockade
      (d) Effect of adrenalectomy
      (e) Effect of sympathectomy

4. Cholinergic mechanisms in the response to hypoglycaemia

      (a) Methods of investigation
      (b) Cholinergic manifestations of the hypoglycaemic reaction
      (c) Gastrointestinal and pancreatic hormone secretion

The regulation of the metabolic recovery from hypoglycaemia is not well understood. The relationships between the endocrine responses, the changes of substrate concentrations in tissues and plasma and the activation of neural mechanisms which are stimulated by glucopenic stress, are complex. Many neural factors can influence the metabolic responses to hypoglycaemia, and both classical divisions of the autonomic nervous system can be implicated. The relative contribution of each division is unclear; studies of neural mechanisms involved in metabolic recovery from hypoglycaemia in animals and man have produced conflicting results, caused in part by considerable species difference.

Before reviewing the separate effects of adrenergic and cholinergic mechanisms on the metabolic recovery from hypoglycaemia, the anatomical organisation and the chemical subdivisions of the autonomic nervous system are described briefly.

1. AUTONOMIC NERVOUS SYSTEM

A. ANATOMICAL ORGANISATION: The autonomic nervous system is influenced by receptors within the brain, with many autonomic functions being controlled by the hypothalamus (Johnson and Spalding, 1974). The peripheral motor pathways of the autonomic nervous system contain pre- and post-ganglionic neurons. The cell bodies of the pre-ganglionic neurons are located in the visceral efferent column of the spinal cord, or the homologous motor nuclei of the cranial nerves (Ganong, 1979). Their axons synapse on the cell bodies of post-ganglionic neurons which then supply visceral effectors. The two classical divisions of the autonomic nervous system are the parasympathetic and sympathetic systems (Fig. 2.1).

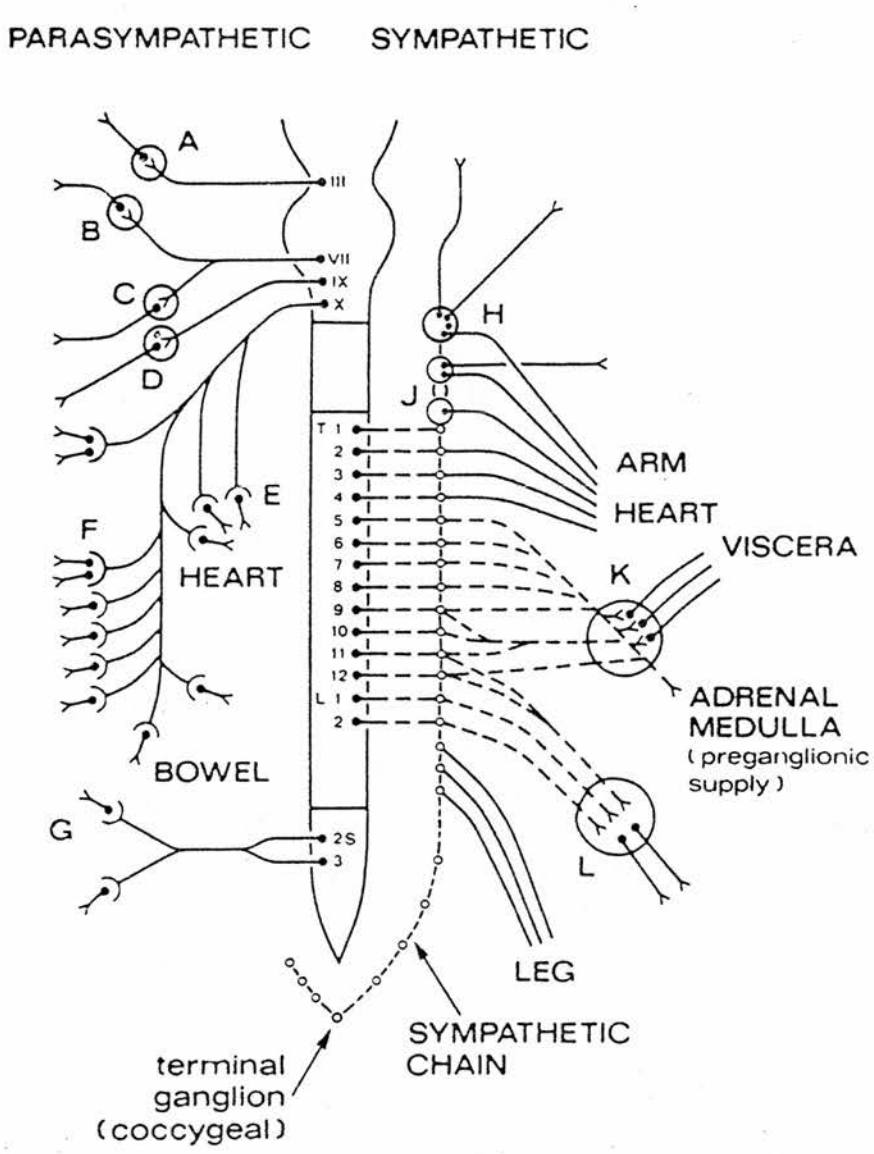

Fig. 2.1 The PARASYMPATHETIC and SYMPATHETIC divisions of the peripheral autonomic nervous system.

PARASYMPATHETIC

from cranial nerves
III, VII, IX, X
and from sacral nerves 2 and 3

A ciliary ganglion
B sphenopalatine ganglion
C submandibular ganglion
D otic ganglion
E vagal ganglion cells in heart wall
F vagal ganglion cells in bowel wall
G pelvic ganglia

SYMPATHETIC

from T1 to L2
pre-ganglionic fibres ----
post-ganglionic fibres ____

H superior cervical ganglion
J middle cervical ganglion and inferior cervical (stellate) ganglion
K coeliac and other abdominal ganglia
L lower abdominal sympathetic ganglia

(a) Parasympathetic division: The cranial outflow of the parasympathetic division supplies the visceral structures in the head via the oculomotor (III), facial (VII) and glossopharyngeal (IX) nerves, and in the thorax and upper abdomen via the vagus (X) nerve. The pelvic viscera are supplied via the sacral outflow (S2 - 3). The pre-ganglionic fibres end on post-ganglionic neurons located on or near the viscera (Fig. 2.1).

(b) Sympathetic division: The axons of the sympathetic pre-ganglionic neurons leave the spinal cord with the ventral roots of the first thoracic (T1) to the second or third lumbar (L2 - 3) spinal nerves. They enter the paravertebral sympathetic ganglion chain where the post-ganglionic neurons are located. The axons of the post-ganglionic neurons pass to viscera in the sympathetic nerves. The post-ganglionic sympathetic nerves to the head originate in the cranial extension of the sympathetic ganglion chain and travel to the effectors via blood vessels.

B. CHEMICAL SUBDIVISIONS: The autonomic nervous system can be subdivided on the basis of the chemical mediator or neurotransmitter released at synaptic junctions, into (a) CHOLINERGIC neurons, in which the transmitter is acetyl choline, and (b) ADRENERGIC neurons, in which the transmitter is noradrenaline.

(a) Cholinergic neurons: These include all pre-ganglionic neurons, anatomical parasympathetic post-ganglionic neurons, and anatomical sympathetic post-ganglionic neurons which innervate sweat glands and produce vasodilatation in blood vessels in skeletal muscle.

(b) Adrenergic neurons: These comprise all remaining post-ganglionic sympathetic neurons. The adrenal medulla is essentially

a modified sympathetic ganglion capable of synthesising adrenaline, in which the post-ganglionic cells have lost their axons and have become specialised for secretion directly into the bloodstream.

C. CHOLINERGIC AND ADRENERGIC RECEPTORS: The properties of the cholinergic receptors differ depending on their location. Muscarine has little effect on autonomic ganglia but mimics the stimulatory action of acetyl choline on smooth muscle and glands. These actions are termed muscarinic, and can be blocked by atropine. In sympathetic ganglia, small amounts of acetyl choline stimulate post-ganglionic neurons, while large amounts block transmission from pre- to post-ganglionic neurons. These effects are unaffected by atropine, but are mimicked by nicotine, and are called nicotinic actions.

The receptors on which noradrenaline and adrenaline act are separated into two categories on the basis of different sensitivities to certain drugs. Adrenergic receptors are designated alpha and beta (Ahlquist, 1948) according to their relative sensitivity to agonists and antagonists, and can be subdivided into $alpha_1$, $alpha_2$, $beta_1$ and $beta_2$ depending on their location (Steer, 1977). The clinically important alpha-effects are vasoconstriction, intestinal relaxation and dilatation of the pupil. Blocking agents include phentolamine, thymoxamine and phenoxybenzamine. The important beta-effects are vasodilatation, especially in muscles, an increase in the rate and force of contraction of the heart, and bronchial relaxation. Blocking agents include propranolol (non-selective) and metoprolol ($beta_1$-selective).

The effects of beta receptor stimulation are produced by activation of adenylate cyclase with a consequent increase in intracellular cyclic AMP. Studies attempting identification of the

intracellular mediator for alpha-adrenergic processes have been inconclusive.

D. HYPOTHALAMIC CONTROL CENTRES IN HYPOGLYCAEMIA: The stimulus within the central nervous system which initiates the autonomic response to hypoglycaemia is probably intracellular glucopenia, but the precise areas which control the sympathetic and parasympathetic responses to hypoglycaemia are unknown. Ablative studies in animals have demonstrated that sub-hypothalamic areas in the medulla oblongata, cervical and thoracic spinal cord may initiate an adrenal medullary response to hypoglycaemia in the presence of surgical decentralisation (Crone, 1965; Goldfien, 1966), but at present there is no evidence for the existence of similar centres in man. Electrical stimulation experiments in the rat have suggested the existence of a centre in the ventromedial nucleus of the hypothalamus which activates the adrenergic response to hypoglycaemia (Frohman and Bernardis, 1971). Stimulation of the dorsomedial nuclei and posterior hypothalamic areas produces increased secretion of catecholamines from the adrenal medulla (Goldfien and Ganong, 1962), and the descending pathways from the hypothalamus to the lateral cell columns of the spinal cord (T1 - L2) have been identified for the sympathetic division (Johnson and Spalding, 1974). However, although a relationship has been identified between the hypothalamus and sympathetic (adrenergic) function there is very little evidence for a "parasympathetic" centre within the hypothalamus (Ganong, 1979), and little is known about the central connections of the parasympathetic system in man.

There is limited evidence for the existence of central adrenergic and cholinergic receptors, located within the hypothalamus, which are

implicated in the activation of autonomic neural function in response to hypoglycaemia. Alpha and beta-adrenergic receptors have been described within the central nervous system (Frohman and Stachura, 1975), and control of the secretion of some anterior pituitary hormones which are released in response to hypoglycaemia (for example ACTH), appears to be mediated, at least in part, by noradrenergic mechanisms within the hypothalamus. The secretion of corticotrophin-releasing hormone (CRH) in rats, dogs and possibly man, is decreased by the action of noradrenaline on alpha-adrenergic receptors within the hypothalamus, thus controlling the secretion of ACTH (Weiner and Ganong, 1978). In addition to central noradrenergic inhibition of CRH, in the murine hypothalamus there is evidence to implicate a cholinergic mechanism in the regulation of CRH secretion (Jones et al., 1976). A similar noradrenergic system, mediated via alpha-adrenergic receptors, can increase the secretion of growth hormone in rats, dogs and in man (Weiner and Ganong, 1978).

2. METHODS OF INDUCING GLUCOPENIA

Although alcohol has been used to induce hypoglycaemia in normal human subjects after a prolonged fast (Turner et al., 1973a), this is not a practical method for numerous or rapid studies of metabolic recovery from hypoglycaemia, nor can it induce a consistent or profound degree of hypoglycaemia. Two methods of inducing acute glucopenia are commonly employed to investigate metabolic changes in response to hypoglycaemia.

(a) Insulin: hypoglycaemia in man is usually induced by the administration of short-acting insulin by intravenous injection in a

dose of 0.1 to 0.15 units/kg body weight. This usually lowers blood glucose to hypoglycaemic levels within 20 to 40 minutes and is widely used as a test of the hypothalamic-pituitary-adrenal axis (Greenwood et al., 1966).

(b) 2-deoxy-D-glucose (2-DG): intracellular glucopenia can be induced by the administration of this glucose analogue which is not metabolised, but acts as a competitive inhibitor of glucose. In normal subjects the intravenous infusion of 2-DG produces hyperglycaemia, fatty acid mobilisation, enhanced excretion of catecholamines and the release of growth hormone (Grasso et al., 1968). There is also a marked rise of the plasma concentrations of lactate and cortisol (Brodows et al., 1973). This glucose analogue is an effective inhibitor of insulin secretion (Goldsmith et al., 1970) and therefore allows assessment of several metabolic parameters without the effect of a concomitant change in insulin secretion and plasma insulin concentrations. It is important to appreciate however that 2-DG infusion produces a greater hypoglycaemic stress than exogenous insulin (Young and Landsberg, 1977), and the possibility that 2-DG and insulin are fundamentally different stimuli cannot be excluded.

3. ADRENERGIC MECHANISMS IN THE RESPONSE TO HYPOGLYCAEMIA

(a) Methods of investigation: Investigations of the role of adrenergic mechanisms and the sympatho-adrenal system in the metabolic and hormonal responses to hypoglycaemia have utilised different types of experimental approach:

(i) the measurement of urinary or plasma concentrations of catecholamines in response to hypoglycaemia,

(ii) the effect of pharmacological adrenergic denervation on the metabolic recovery from hypoglycaemia using ganglion-blocking drugs or drugs which specifically blockade alpha or beta-adrenergic receptors,

(iii) the effect of hypoglycaemia on adrenalectomised animals or human subjects, and

(iv) the effect of hypoglycaemia on sympathectomised animals or man.

Pharmacological interruption of sympathetic activity at the level of peripheral ganglia using drugs such as hexamethonium should prevent both adrenal medullary and post-ganglionic sympathetic responses to hypoglycaemia, but the dose used may not achieve complete blockade (Billington et al., 1954; DiSalvo et al., 1956), and the degree of pharmacological blockade cannot be quantified precisely. Furthermore, certain ganglionic and adrenergic-blocking agents actually have intrinsic sympathomimetic activity (Dollery et al., 1969).

Hypoglycaemia is the only state which is recognised to produce preferential secretion of adrenaline, which becomes the predominant circulating catecholamine. This implies a selective activation of the adrenal medulla. Denervation of the adrenal glands or adrenalectomy abolishes the acute release of adrenaline in response to hypoglycaemia (Goldfien, 1966). In adrenalectomised subjects the primary source of circulating noradrenaline is from the peripheral sympathetic nerve endings. The study of adrenalectomised animals or man therefore permits an examination of the sympathetic response to hypoglycaemia in the absence of adrenomedullary catecholamines.

Patients who have undergone bilateral splanchnicectomy and surgical removal of the sympathetic chain effectively have adrenal

denervation and a peripheral sympathectomy (French and Kilpatrick, 1955). Traumatic lesions of the cervical spinal cord in man, producing functional transection of the major motor and sensory tracts with tetraplegia, are associated with a complete pre-ganglionic sympathectomy when the lesion is above the first thoracic segment (Fig. 2.1). This provides an excellent model for the evaluation of adrenergic mechanisms in vivo. The adrenal glands and the peripheral sympathetic nervous system are disconnected simultaneously from the CNS, and the pre-ganglionic site of the lesion should preclude the possibility of denervation hypersensitivity of peripheral adrenergic receptors.

(b) Secretion of catecholamines: After the classical experiments of Cannon et al., (1924) and Houssay et al., (1924) which implicated the activation of adrenal medullary secretion in the response to hypoglycaemia, nearly thirty years elapsed before an increase in the urinary excretion of catecholamines could be measured reliably following hypoglycaemia in man (Von Euler and Luft, 1952). The subsequent development of satisfactory assays for plasma adrenaline and noradrenaline enabled the demonstration of the dramatic rise in plasma catecholamines which occurs in response to hypoglycaemia in man (Vendsalu, 1960; Goldfien et al., 1961; Wallace and Harlan, 1965; Christensen et al., 1975; Garber et al., 1976). A close correlation has been demonstrated between the degree of hypoglycaemia and the rise of plasma adrenaline, which can increase by 25 to 50 times from basal levels (Christensen et al., 1975). Plasma noradrenaline hasbeen shown to increase by two to threefold (Christensen et al., 1975; Garber et al., 1976).

The increase in plasma noradrenaline concentration is derived from the adrenal medulla in addition to release from the sympathetic

nerve endings (Bloom et al., 1975). The measurement of urinary or plasma catecholamines therefore has a limited value in differentiating the separate contributions of adrenal medullary secretion and peripheral sympathetic neural activity in response to hypoglycaemia.

(c) Effect of pharmacological blockade: Ganglionic blockade with drugs such as hexamethonium has been shown to reduce the rises of blood lactate and plasma free fatty acids which are stimulated by hypoglycaemia in man (Billington et al., 1954; DiSalvo et al., 1956; Werk et al., 1961), but with the exception of one study (Laurence and Stacey, 1952), no significant impairment of blood glucose recovery was observed. In these studies the changes in pulse rate and blood pressure associated with hypoglycaemia were absent, and sweating was not observed (Laurence and Stacey, 1952).

The introduction of propranolol, a non-selective beta-adrenergic blocking agent, allowed a re-examination of the specific contribution of adrenergic receptors to metabolic recovery from hypoglycaemia. In dogs blood glucose recovery from insulin-induced hypoglycaemia was not impaired by propranolol, but the addition of simultaneous alpha-adrenergic blockade with phenoxybenzamine produced a marked delay in blood glucose recovery (Cowell and Hetenyi, 1969). One study of the effect of adrenergic blockade in normal man demonstrated a normal blood glucose recovery from acute hypoglycaemia during the infusion of propranolol or phentolamine, but the effect of both drugs in combination was not studied (Walter et al., 1974). However most human studies have shown that beta-adrenergic blockade with propranolol does impair blood glucose recovery from hypoglycaemia, and virtually abolishes the rise of free fatty acids, glycerol (Abramson et al., 1966; Blackard and Heidingsfelder, 1968) and blood lactate (Abramson and Arky, 1968). The effect of beta blockade

on blood glucose recovery from hypoglycaemia has been confirmed in both normal (Deacon and Barnett, 1976; Davidson et al., 1977) and diabetic man (Deacon et al., 1977; Lager et al., 1979). There is no clear reason for the discrepancy between the findings of Walter et al., (1974) and the other studies of non-selective beta blockade on blood glucose recovery from hypoglycaemia, but it may reside in methodological differences.* The administration of the $beta_1$ (selective) blocking agents, metoprolol (Davidson et al., 1977) and atenolol (Deacon and Barnett, 1976) did not delay blood glucose recovery significantly in normal subjects, and metoprolol did not impair blood glucose recovery from hypoglycaemia in patients with insulin-dependent diabetes (Lager et al., 1979).

Propranolol produces a marked bradycardia and a rise in both the systolic and diastolic blood pressures during hypoglycaemia in normal subjects (Lloyd-Mostyn and Oram, 1975) and these effects were much less pronounced with metoprolol (Davidson et al., 1977; Lager et al., 1979). This difference in action may result from the unopposed action of alpha receptors during the administration of propranolol thus encouraging vasoconstriction, whereas with metoprolol the vasodilating $beta_2$ receptors may still be stimulated by catecholamines.

The effect of non-selective beta blocking agents on blood glucose recovery from hypoglycaemia may operate via inhibition of muscle glycogenolysis and adipose tissue lipolysis, both of which are mediated by beta receptors (Abramson and Arky, 1968). The diminished supply of glucogenic substrates such as lactate, alanine and glycerol would presumably decrease the rate of gluconeogenesis in response to hypoglycaemia. Hepatic glycogenolysis was considered to be mediated via an alpha-adrenergic effect (Abramson and Arky, 1968; Blackard and Hubbell, 1970) and should therefore be unaffected by

propranolol. However, recent studies utilising glucose clamp techniques (Deibert and Defronzo, 1979) or somatostatin infusions (Rizza et al., 1979a; Rizza et al., 1980), which prevent changes in circulating substrate or hormone concentrations, have demonstrated that infusions of adrenaline stimulate glucose production and inhibit glucose clearance in man predominantly by beta-adrenergic receptor mechanisms. The hyperglycaemic response to catecholamines may therefore be mediated primarily by beta-adrenergic receptors, although the putative involvement of a mixed population of alpha, beta and unclassified receptors has also been proposed (Kuo et al., 1977; Saitoh and Ui, 1976).

(d) Effect of adrenalectomy: Adrenalectomy has had no demonstrable effect on blood glucose recovery from insulin-induced hypoglycaemia in man (Ginsburg and Paton, 1956; Von Euler et al., 1961; Ensinck et al., 1976; Brodows et al., 1976). However the administration of 2-DG to induce intracellular glucopenia in adrenalectomised human subjects did not produce the usual degree of hyperglycaemia observed in normal subjects (Wegienka et al., 1966; Brodows et al., 1975). and the elevationsof blood lactate and plasma free fatty acids were abolished (Brodows et al., 1975). Several studies have examined the effect of administering 2-DG to adrenalectomised rats and dogs, but while the increase in blood glucose was diminished consistently, the plasma free fatty acid response was variable (Young and Landsberg, 1977). Hypoglycaemia in adrenalectomised subjects is not associated with a change in pulse rate and blood pressure (Ginsburg and Paton, 1956), which suggests that the haemodynamic changes observed in normal subjects during hypoglycaemia are related to the release of adrenal medullary catecholamines.

(e) Effect of sympathectomy: In adreno-medullectomised rats which were treated with reserpine to produce a functional (total) sympathectomy, insulin-induced hypoglycaemia was more pronounced than in normal rats, and blood glucose recovery was severely impaired (Sacca et al., 1977). Early investigations of human subjects, who had undergone thoracolumbar sympathectomy for hypertension, showed no evidence of insulin sensitivity or impairment of blood glucose recovery from hypoglycaemia (Simeone and Vavoudes, 1948; French and Kilpatrick, 1955). Blood glucose recovery was also normal in tetraplegic subjects in whom cervical cord lesions had produced a complete pre-ganglionic sympathectomy (Palmer et al., 1976; Brodows et al., 1976), and both basal insulin secretion and glucose-mediated insulin release were also normal in these subjects (Brodows et al., 1974).

By contrast the administration of 2-DG to sympathectomised human subjects to induce glucopenic stress, was characterised by no detectable rise in glucose compared with the hyperglycaemia observed in normal subjects (Brodows et al., 1973). There was also no rise in plasma catecholamines, free fatty acids or lactate compared with the pronounced changes which normally are observed. This was consistent with the absence of a FFA rise when 2-DG was administered to sympathectomised rats (Richardson and Hokfelt, 1964). The severity of the glucopenic stress induced by 2-DG is probably not comparable to insulin-induced hypoglycaemia, and this may account in part for the apparent discrepancies between the metabolic responses to either form of glucopenic stimulus either in animals or in human subjects with altered sympatho-adrenal function.

A fall in both the systolic and diastolic blood pressure was demonstrated during hypoglycaemia in patients with a bilateral

sympathectomy (T7 - L3), although normal vasodilatation remained present in the upper limbs where the innervation was intact (French and Kilpatrick, 1955). A preceding unilateral cervical sympathectomy has been shown to decrease the blood flow in the hand of the affected side, and prevent the vasodilatation which is observed in normal subjects (Allwood et al., 1957; Middleton and French, 1974). The increased hand blood flow associated with hypoglycaemia is thought to result from a release of vasocontrictor tone. Acute hypoglycaemia thereby induces vasodilatation in the hand which usually counteracts and obscures any simultaneous vasoconstriction caused by the release of adrenaline (Middleton and French, 1974).

These studies indicate that circulating catecholamines promote the haemodynamic changes which occur in response to hypoglycaemia, and adrenergic mechanisms mediate the mobilisation of free fatty acids and lactate. However, activation of the sympatho-adrenal system does not appear to be essential for blood glucose recovery from hypoglycaemia in man.

4. CHOLINERGIC MECHANISMS IN THE RESPONSE TO HYPOGLYCAEMIA

Most studies of acute hypoglycaemia have investigated the adrenergic manifestations of the hypoglycaemic reaction and the endocrine responses related to activation of the sympatho-adrenal system. There is evidence however for the production of widespread autonomic neural activity in response to hypoglycaemia which involves cholinergic mechanisms in both the sympathetic and parasympathetic divisions.

(a) Methods of investigation: The role of cholinergic innervation in the metabolic and hormonal responses to hypoglycaemia

has been investigated by:

(i) pharmacological blockade using atropine or related drugs, and

(ii) ablation studies producing parasympathetic denervation (mainly vagotomy)

It is extremely difficult to achieve complete cholinergic blockade with atropine in human subjects without administering doses which will produce toxic side-effects, but the relative effect of partial cholinergic denervation can be demonstrated.

(b) Cholinergic manifestations of the hypoglycaemic reaction: Haemodynamic changes: The existence of a "parasympathetic response" to hypoglycaemia was suggested by the description of the occasional occurrence of bradycardia and mild hypotension, which is sometimes accompanied by nausea and vomiting in some patients with insulin-dependent diabetes (Sussman et al., 1963). This was observed to precede the typical "sympathetic response" which characterises the hypoglycaemic reaction. This evidence was inconstant and circumstantial, but bradycardia which could be abolished by atropine has been described following hypoglycaemia in normal subjects under beta-adrenergic blockade with propranolol (Lloyd-Mostyn and Oram, 1975). A late bradycardia has been observed in sympathectomised subjects which occurred when the blood pressure was lower than basal values (French and Kilpatrick, 1955), and thus would preclude the induction of bradycardia by a baroreceptor mechanism. These haemodynamic observations suggest a primary vagal activation in response to hypoglycaemia.

Salivation: Profuse salivation was noted to be a feature of the hypoglycaemic reaction in sympathectomised cats (Dworkin, 1931b), and toxic quantities of insulin produced marked salivation in dogs

(Ponirowsky and Ischunina, 1929). The flow rate of parotid saliva is controlled by cholinergic parasympathetic activity in the glossopharyngeal nerves (Garrett, 1975), and increased salivation has been demonstrated to coincide with the hypoglycaemic reaction in man (Corrall et al., 1976).

Sweating: Sweating is a constant feature of the hypoglycaemic reaction in normal subjects. It is mediated via cholinergic sympathetic activity and not by circulating catecholamines. Sweating persists after adrenal denervation (French and Kilpatrick, 1955) and after adrenalectomy (Ginsburg and Paton, 1956). It is abolished by atropine (given systemically or locally by iontophoresis), and after ulnar nerve blockade sweating was absent in the anaesthetised area (French and Kilpatrick, 1955).

Vasodilatation: Hypoglycaemia provokes increased cholinergic vasodilator activity in skeletal muscle (Allwood and Ginsburg, 1959), and an increase in blood flow was demonstrated in the forearm and calf of normal human subjects both in neurologically intact and in sympathectomised limbs in response to hypoglycaemia (Allwood et al., 1957). The unilateral infusion of intra-arterial atropine during insulin-induced hypoglycaemia significantly reduced the forearm vasodilator response, indicating the role of cholinergic sympathetic fibres which produce vasodilatation in skeletal muscle.

(c) Gastro-intestinal and pancreatic hormone secretion: Insulin-induced hypoglycaemia has been a standard stimulatory test of vagally-mediated gastric acid secretion for many years, thus providing a suitable measure of the efficacy of surgical vagotomy. In normal subjects, pancreatic polypeptide is secreted in response to hypoglycaemia and this is abolished by vagotomy (Schwartz et al., 1976; Adrian et al., 1977; Floyd et al., 1977) and diminished by atropine (Schwartz et al., 1978). Pancreatic polypeptide is also released after

stimulation of the vagus nerves in anaesthetised pigs, and this response can be inhibited both by hexamethonium and by atropine, but it is unaffected by alpha or beta-adrenergic blockade (Schwartz et al., 1978). It is apparent therefore that the secretion of pancreatic polypeptide, particularly in response to hypoglycaemia, is mediated by vagal cholinergic stimulation. In dogs a direct stimulatory influence of vagal activation on gastrin secretion has been demonstrated, but in man the vagal contribution to gastrin secretion is defined less clearly (Young and Landsberg, 1977).

The effect of hypoglycaemia on glucagon secretion has been studied in animals and man. In calves, section of the splanchnic nerves or the administration of atropine alone had no effect on the blood glucose recovery from hypoglycaemia, and caused only a delay in the peak response of pancreatic glucagon (Bloom et al., 1974a). Section of the splanchnic nerves combined with atropine blockade markedly reduced insulin tolerance in these animals, producing severe and prolonged hypoglycaemia. The rise of plasma pancreatic glucagon virtually was abolished. Section of both vagus nerves in calves also reduced insulin tolerance and the pancreatic glucagon response to hypoglycaemia (Edwards and Bloom, 1978). Stimulation of the thoracic vagus nerves produced a significant release of glucagon in adrenalectomised calves with co-existing splanchnic nerve section (Bloom et al., 1974a). When 2-DG was used as the hypoglycaemic stimulus in normal calves, an abrupt rise of blood glucose, glucagon, insulin and pancreatic polypeptide was observed (Edwards and Bloom, 1978). Section of the splanchnic nerves plus atropine administration again abolished the rise in pancreatic glucagon (and pancreatic polypeptide) in response to 2-DG, and the normal rise in blood glucose was greatly diminished.

In normal human subjects the administration of atropine produced a small decrease in plasma pancreatic glucagon after an overnight fast, and the normal rise of plasma glucagon after stimulation by arginine was reduced (Bloom et al., 1974b). The effect of insulin-induced hypoglycaemia in patients who had undergone a truncal (total) vagotomy for duodenal ulcer, was compared with that in patients who had received a selective vagotomy. The normal rise of plasma pancreatic glucagon was preserved following selective vagotomy, but appeared to be decreased moderately after truncal vagotomy (Bloom et al., 1974b).

There is some circumstantial evidence for a possible vagal mediation of glucagon secretion following hypoglycaemia in patients with insulin-dependent diabetes. Although pancreatic glucagon has been shown to be released in response to arginine in diabetic patients with autonomic neuropathy, glucagon secretion in response to hypoglycaemia is absent (Maher et al., 1977). The sympathetic nervous system does not appear to be necessary for the integrity of the glucagon response to hypoglycaemia in man (Palmer et al., 1976; Ensinck et al., 1976; Walter et al., 1974) and it is therefore implied that the loss of parasympathetic innervation is responsible for the failure of the glucagon response to hypoglycaemia in patients with diabetic autonomic neuropathy. These studies suggest that glucagon release from the pancreas in response to hypoglycaemia is influenced by cholinergic mechanisms via vagal innervation.

Both classical divisions of the autonomic nervous system are involved in the neural mechanisms which initiate or modulate the metabolic recovery to acute hypoglycaemia in man. The relative importance of adrenergic and cholinergic mechanisms to the substrate and hormonal changes which have been described, requires elucidation,

and it is possible that some responses can function independently of neural regulation. The identification in recent years of an extensive and complex peptidergic nervous system which appears to have a significant though incompletely defined role in the regulation of gastro-intestinal, and possibly pancreatic, endocrine function (Polak and Bloom, 1978), introduces a further potential system by which the metabolic response to hypoglycaemia may be regulated. The significance of the peptidergic nervous system to recovery from hypoglycaemia is at present undetermined, and could prove ultimately to be of major importance in the modulation of the hormonal changes which are provoked by acute hypoglycaemia.

PART II EXPERIMENTAL DATA

CHAPTER 3

RATIONALE AND AIMS
OF EXPERIMENTAL INVESTIGATIONS OF HYPOGLYCAEMIA IN MAN

The experimental studies described in succeeding chapters are concerned with the investigation of two areas related to the metabolic recovery from acute hypoglycaemia in man. These can be categorised as:

1. the effect of acute hypoglycaemia on pancreatic beta cell function in response to a subsequent meal,
   and
2. neural control of pancreatic islet cell function and the metabolic and hormonal responses to hypoglycaemia.

This chapter provides a brief introduction to each area of investigation, and outlines the aims of the experimental investigations.

1. THE EFFECT OF ACUTE HYPOGLYCAEMIA ON PANCREATIC BETA CELL FUNCTION IN RESPONSE TO A SUBSEQUENT MEAL

A basic principle of endocrine homeostasis is that hormones are subject to feedback inhibition by a variety of mechanisms, and for many years it was assumed tacitly that insulin secretion is inhibited by hypoglycaemia (Goodner and Porte, 1972). The possible existence of a feedback inhibition of insulin secretion by insulin itself, has been however the subject of several studies and considerable controversy. Assessment of the secretion of endogenous insulin in man during hypoglycaemia has been restricted by an inability to interpret plasma concentrations of immunoreactive insulin, because of the cross-reaction of exogenous mammalian insulin with endogenous human insulin in the insulin radioimmunoassay. Attempts to circumvent this problem have included the induction of hypoglycaemia with alcohol (Turner et al., 1973a), and the use of exogenous insulins which differ from mammalian insulins in their immunological specificity but which possess comparable biological activity. Fish insulin has been used to induce

hypoglycaemia in dogs (Sando et al., 1970) and in man (Turner et al., 1973b), and in both species a fall in endogenous plasma insulin concentrations was demonstrated during hypoglycaemia.

The development of a radioimmunoassay for connecting (C-) peptide (Melani et al., 1970), which is secreted by the pancreatic beta cell in an equimolar concentration with insulin (Horwitz et al., 1975a), provided an accurate index of endogenous insulin secretion in the presence of exogenous insulin (Rubenstein et al., 1969). When porcine insulin was infused intravenously into normal human subjects to induce hypoglycaemia, plasma C-peptide decreased below basal levels, and remained low for 80 minutes after the infusion of insulin had been discontinued (Horwitz et al., 1975b). The delayed recovery of plasma C-peptide following hypoglycaemia indicated that the pancreatic beta cells were releasing neither C-peptide nor insulin at their basal rate of secretion, and this prolonged suppression of endogenous insulin secretion continued despite the restoration of blood glucose to its preceding basal value. Similar findings in response to hypoglycaemia have been reported both in normal and in thyrotoxic subjects (Shima et al., 1976), and the demonstration of a failure of insulin-induced hypoglycaemia to suppress plasma C-peptide is a useful diagnostic test for insulinoma (Service et al., 1977).

The effect of hypoglycaemia on the capacity of the pancreatic beta cell to secrete insulin in response to the subsequent ingestion of food has received little attention. Somogyi (1951) described impaired tolerance to oral glucose ingested 50 minutes after insulin administration, but this was attributed to the presence of hormones that are released in response to hypoglycaemia and which antagonise the actions of insulin. Ohgawara et al., (1973) infused fish insulin into normal subjects for three to eight hours, and observed a

diminished insulin secretory response to subsequent loading with intravenous glucose. A similar infusion of fish insulin for one hour did not produce this effect on insulin secretory capacity.

The response of the pancreatic beta cell to the ingestion of food following hypoglycaemia has not been reported previously. The experimental studies which are described in chapters 4 and 5 attempt to answer the following questions:

(a) What is the effect of hypoglycaemia on pancreatic beta cell function in response to a subsequent meal?

(b) If the pattern of insulin secretion following hypoglycaemia is abnormal in response to a meal, what is the possible mechanism?

2. NEURAL CONTROL OF PANCREATIC ISLET CELL FUNCTION AND THE METABOLIC AND HORMONAL RESPONSES TO HYPOGLYCAEMIA

Acute hypoglycaemia is associated with stimulation of the pancreatic alpha cells to secrete glucagon (Gerich et al., 1974) and a concurrent prolonged suppression of the secretion of insulin by the pancreatic beta cells (Horwitz et al., 1975b; Service et al., 1977). By studying the neural regulation of pancreatic islet cell function during hypoglycaemia, it is possible to identify the contribution of adrenergic and cholinergic mechanisms which influence or regulate the secretion of insulin and pancreatic glucagon.

The pancreatic islets receive an extensive supply of efferent sympathetic and parasympathetic nerve fibres (Bencosme, 1959; Woods and Porte, 1974). Studies in animals have shown that stimulation of the parasympathetic innervation leads to the secretion of insulin, but under resting conditions, parasympathetic influences have little effect on insulin release, which is inhibited tonically by sympathetic

alpha-adrenergic activity (Woods and Porte, 1974). The role of the autonomic innervation of the pancreatic islets in the control of glucagon secretion is not defined clearly, but both the sympathetic and parasympathetic divisions appear to contribute to the maintenance of glucagon secretion in the basal state (Edwards and Bloom, 1978).

Despite attempts to elucidate the putative neural mechanisms which control pancreatic islet cell function, no human studies have examined previously the effect of combined sympathetic and parasympathetic blockade on the recovery from insulin-induced hypoglycaemia. Tetraplegic subjects, who have a complete pre-ganglionic sympathectomy, provide a suitable human model to study the effects of adrenergic denervation on the metabolic recovery from hypoglycaemia and on pancreatic islet cell secretion. The administration of atropine to these tetraplegic subjects, producing concurrent cholinergic blockade, allows an assessment of cholinergic mechanisms in this situation. The relative contribution of the principal counterregulatory hormones to metabolic recovery from hypoglycaemia can also be examined. Studies of acute hypoglycaemia in tetraplegic subjects with and without the administration of atropine are described in Chapters 6, 7 and 8.

The experimental protocols were approved by the Medical Ethics Committee of the Western General Hospital, Edinburgh, where the studies were performed. Informed consent for each study was obtained from all subjects. The tetraplegic patients were recruited for these investigations from the Spinal Injuries Unit, Edenhall Hospital, Musselburgh, East Lothian.

CHAPTER 4

THE EFFECT OF ACUTE HYPOGLYCAEMIA ON THE PANCREATIC BETA CELL RESPONSE TO A MEAL IN MAN

Chapter 4

The effect of acute hypoglycaemia on the pancreatic beta cell response to a meal in man

Introduction

Subjects and Methods:

(I) Hypoglycaemia study
(II) Control (fasting) study

Measurement of sweat production

Results:

Clinical manifestations of hypoglycaemia
Blood glucose
Plasma C-peptide
Plasma insulin

Discussion

The development of a specific radioimmunoassay for human C-peptide has enabled the study of endogenous insulin secretion *in vivo* during hypoglycaemia induced by the administration of exogenous insulin (Horwitz et al., 1975a). The demonstration in man of impaired tolerance to oral glucose after insulin administration (Somogyi, 1951) was complemented by the observation that the insulin secretory response to a glucose load was decreased after the prolonged infusion of fish insulin (Ohgawara et al., 1973). These observations have been extended by examining the response of the pancreatic beta cell to a meal after preceding hypoglycaemia in man.

SUBJECTS AND METHODS

Eleven healthy subjects (9 male, 2 female), age range 20 - 29 years (mean 23.8 years),were studied supine. All subjects were within ten per cent of their ideal body weight (mean 96 per cent, range 91 - 102 per cent). Nine of the subjects (nos. 3 - 11) were studied on two separate occasions with an interval of at least one week. The first two subjects (nos. 1 and 2) participated only in the hypoglycaemia study.

(I) Hypoglycaemia study: After an overnight fast crystalline beef insulin, 0.15 units/kg body weight, was administered as a bolus by rapid intravenous injection, and serial blood samples were taken via an indwelling teflon cannula for estimation of blood glucose (Hill and Kessler, 1961), plasma immunoreactive insulin,assayed against a human insulin standard, RD10 (Ashby and Speake, 1975),and plasma C-peptide immunoreactivity using an anti-synthetic human C-peptide guinea pig serum, M1230 (Heding, 1975). Blood samples were withdrawn in the basal state and at frequent intervals for 210 minutes after the injection

of insulin. Nine of the subjects (nos. 3 - 11) were then given a standard mixed meal containing 30 G. protein, 85 G. carbohydrate and 40 G. fat, which was consumed within 15 minutes. Blood sampling was continued for a further 120 minutes.

(II) Control (fasting) study: Nine subjects (nos. 3 - 11) were restudied after an interval of at least one week. The standard meal was given following an overnight fast plus an equivalent period of fasting without the administration of insulin. Similar measurements of blood glucose, plasma insulin and C-peptide were made from serial blood samples, commencing immediately prior to ingestion of the standard meal.

Pulse and blood pressure weremeasured at two minute intervals in all subjects until 60 minutes after the administration of insulin, and thereafter at 5 minute intervals.

The timing of the onset of the hypoglycaemic reaction in each subject coincided with an increase in pulse rate and the appearance of neuroglycopenic symptoms. In man a wide individual variation exists in the time of onset of the hypoglycaemic reaction after insulin administration, and in this study, it varied from 20 to 30 minutes after insulin. To account for the individual biological variation in the timing of blood samples during the recovery from hypoglycaemia, the clock timer was reset at the time of the onset of the hypoglycaemic reaction in each study, to ensure that subsequent blood sampling was timed from this point. In all figures which depict the changes of the metabolic parameters measured in these studies, the mean values at the time of the hypoglycaemic reaction are represented at 30 minutes after insulin.

Measurement of sweat production: The rate of sweat production was measured using a micro-hygrometer in four subjects. This was determined

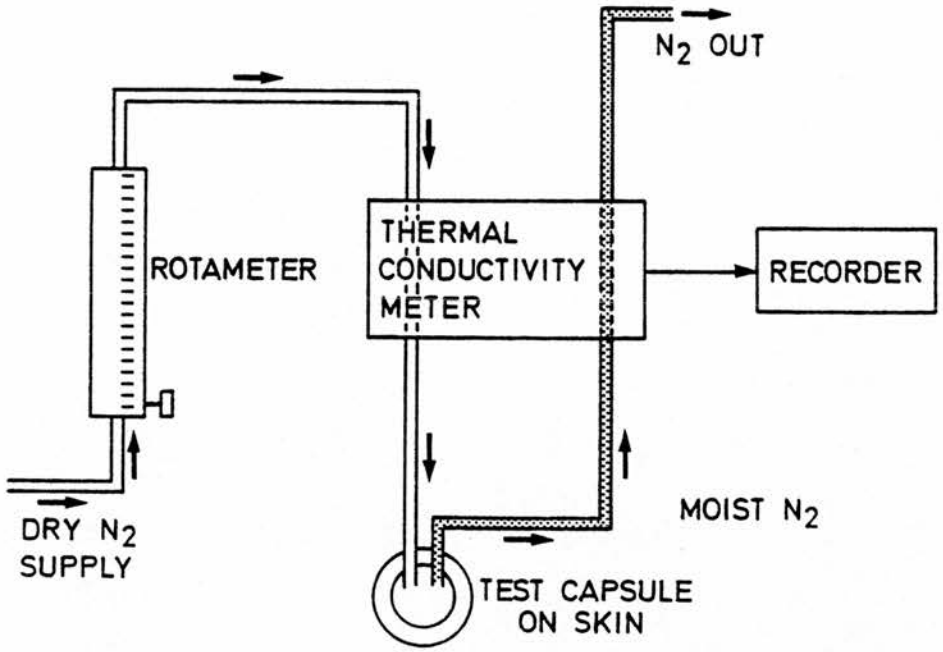

Fig. 4.1 Diagram of apparatus used for the measurement of sweat production.

by evaporating the sweat in a stream of dry nitrogen and measuring the moisture content of the resulting mixture (unpublished method). The apparatus is represented in diagrammatic form in Fig. 4.1, and consists of three main parts:

(1) A flow meter, with which a measured flow of 800 ml/min is maintained by means of a regulatory valve on a cylinder of dry nitrogen.

(2) A test capsule (area 9.6 cm^2) placed on the skin through which dry nitrogen passes.

(3) A thermal conductivity meter that measures the difference in conductivity between the dry gas going to the cell and the moist gas leaving it. This is based on the Gow-Mac Unit 9454 with W-2 elements (Gow-Mac Instruments, Shannon, Eire).

The capsule was strapped over the right hypochondrium at the start of the experiment. Recordings were made on a servoscribe RE511.20 machine (MacFarlane Robson Ltd., Blaydon-on-Tyne, Co. Durham, NE21 4LT). The area under the curve on the chart gives a measure of total sweat production. The meter was calibrated in mg of water released $cm^{-2}min^{-1}$ by injecting water on to a filter paper on a plate maintained at 45^{o} in a modified chamber. The method has a precision of 4.9 per cent (S.E. of repeated observations, n = 4).

Statistical methods: Results are expressed as mean $\pm$ one standard error of the mean (SEM). Statistical significance was determined using Student's t test for paired data.

RESULTS

Clinical manifestations of hypoglycaemia: All subjects experienced the typical symptoms of hypoglycaemia between 20 and 30 minutes after

TABLE 4.1

ACUTE HYPOGLYCAEMIA IN NORMAL SUBJECTS

SUBJECT NO.	AGE AND SEX	ONSET OF HYPOGLYCAEMIC REACTION: TIME AFTER INSULIN (min)	BLOOD GLUCOSE NADIR (mmol/l)	BASAL PULSE RATE (beats/min)	MAXIMUM PULSE RATE (beats/min)
1	27 M	30	1.5	68	88
2	23 F	25	1.2	94	116
3	23 M	24	1.3	80	104
4	20 F	25	1.5	66	88
5	23 M	23	1.0	64	100
6	22 M	27	1.4	78	100
7	29 M	23	0.7	76	92
8	23 M	24	1.1	56	76
9	22 M	20	1.1	60	84
10	21 M	20	0.8	64	92
11	29 M	25	1.1	64	84

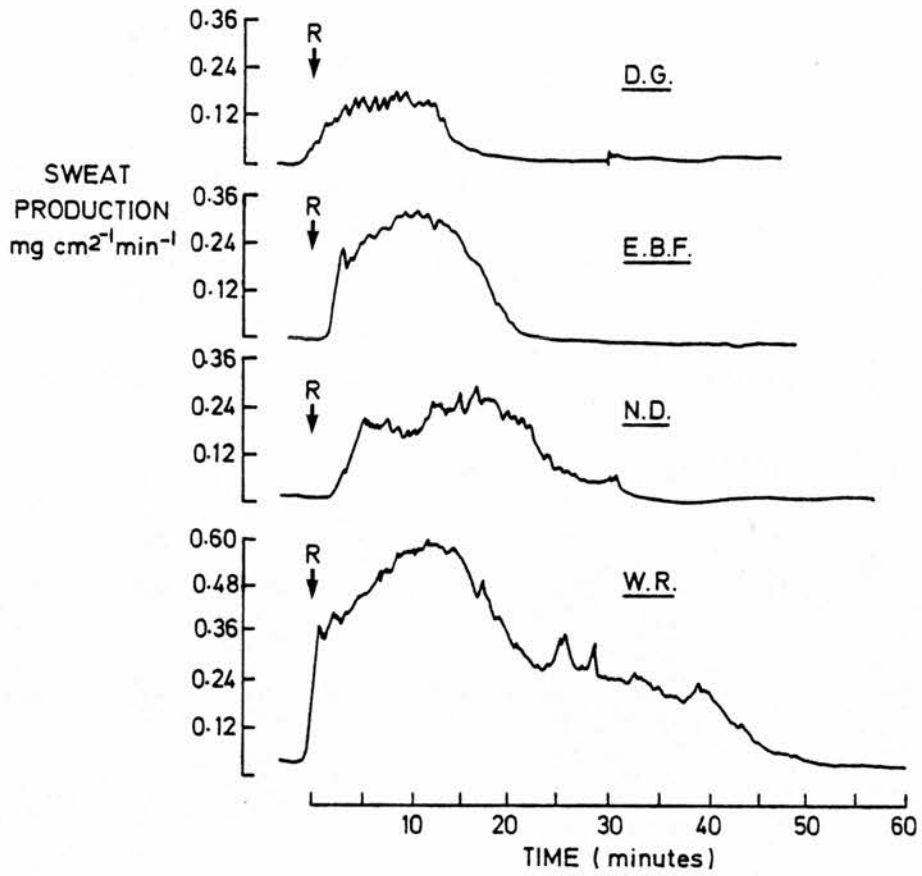

Fig. 4.2 Pattern of sweating response during hypoglycaemia in four normal subjects. The onset of the hypoglycaemic reaction is signified by R, and the time from R is recorded on the horizontal axis.

TABLE 4.2

SWEATING RESPONSE TO HYPOGLYCAEMIA

SUBJECT	TOTAL SWEAT mg cm^{-2}	TIME OF ONSET OF SWEATING (R = Reaction)	PEAK (min)	DURATION (min)
1	1.92	R - 1½ min	6	20
2	4.2	R + 1½ min	8	25
3	3.9	R + 1½ min	15	35
4	14.0	Coincidental	8	55

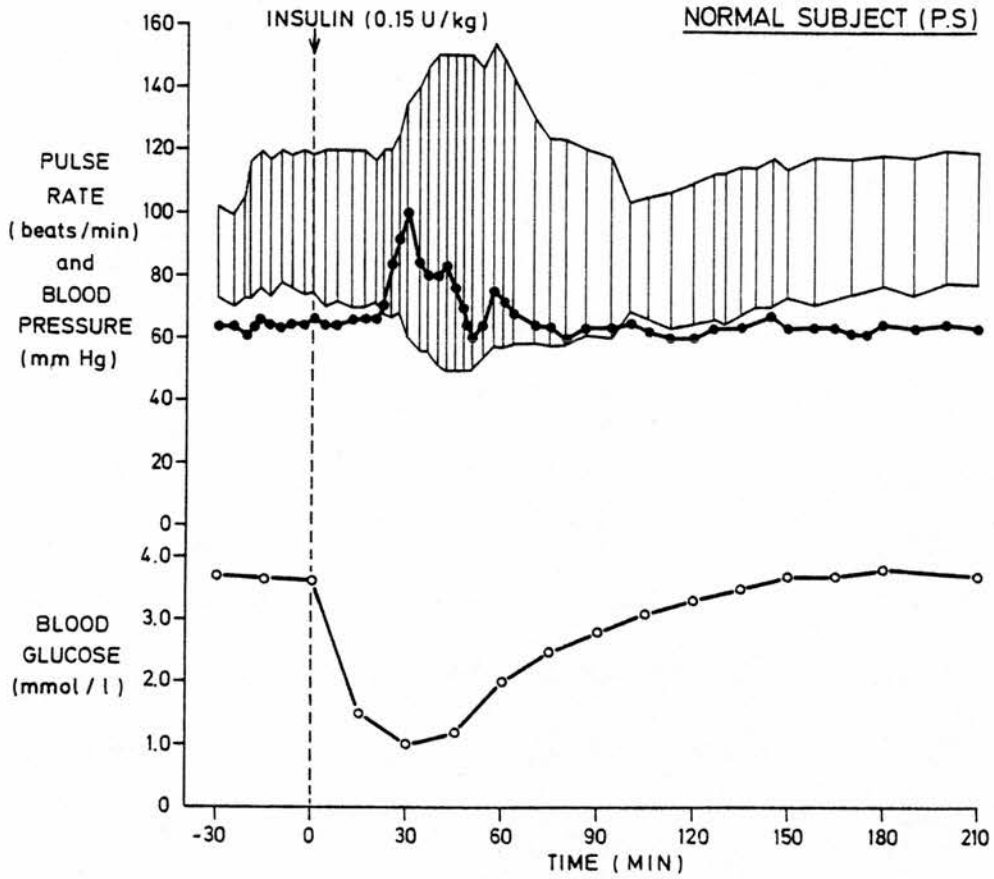

Fig. 4.3 Haemodynamic changes in a normal subject. The changes in pulse rate and blood pressure in response to hypoglycaemia are shown, with the concurrent blood glucose concentrations following injection of insulin.

the injection of insulin (mean 24 min). Individual times are shown in Table 4.1. Symptoms included profuse sweating and mild neuroglycopenia with a sensation of hunger and mild drowsiness. The changes in sweat production in four subjects are shown in Fig. 4.2 and the total sweat produced (mg cm^{-2}) is shown in Table 4.2. The onset of sweating was sudden in all subjects and occurred within 1.5 minutes of the reaction. The total duration of increased sweating varied between 20 and 55 minutes.

The haemodynamic changes of hypoglycaemia were exhibited by all subjects. A transient tachycardia coincided with the nadir of the blood glucose and lasted for only 10 to 15 minutes. The increase in pulse rate of individual subjects is shown in Table 4.1. An increase in systolic blood pressure was accompanied by a fall in diastolic pressure with a consequent widening of pulse pressure in all subjects. A typical example of these haemodynamic changes is shown in Fig. 4.3 (subject no. 5).

Blood glucose: The mean blood glucose concentrations in both the hypoglycaemia and control studies are shown in Table 4.3. In the hypoglycaemia study the mean fasting blood glucose fell from 3.9 $\pm$ 0.1 to 1.2 $\pm$ 0.1 mmol/l at the time of the acute hypoglycaemic reaction, and regained the fasting level by 150 min after the injection of insulin (Fig. 4.4). Following the meal, the mean blood glucose rose to a peak of 8.4 $\pm$ 0.3 mmol/l at 60 min and was still raised (7.5 $\pm$ 0.3 mmol/l) at 120 min post-prandially.

In the control study the mean blood glucose of the nine subjects studied reached a peak value of only 5.1 $\pm$ 0.2 mmol/l at 30 min after the meal, and regained the fasting level (3.6 $\pm$ 0.3 mmol/l) by 60 min post-prandially (Table 4.4). The difference in the mean blood

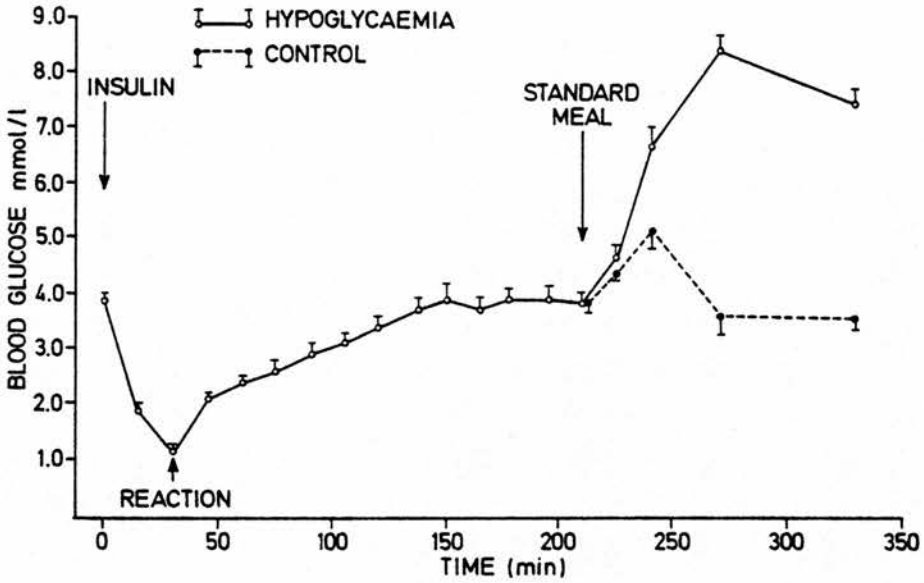

Fig. 4.4 Blood glucose concentration (mean ± SEM) following injection of insulin, and in response to a subsequent meal (hypoglycaemia study), and in response to a meal alone after an equivalent period of fasting (control study) (n = 9).

TABLE 4.3

MEAN BLOOD GLUCOSE, PLASMA C-PEPTIDE AND INSULIN IN NORMAL SUBJECTS.

Insulin administered at 0 min, and meal consumed at 210 min.

	TIME (min)	BLOOD GLUCOSE (mmol/l) (mean ± S.E.M.)	PLASMA C-PEPTIDE (nmol/l) (mean ± S.E.M.)	PLASMA INSULIN (mU/l) (mean ± S.E.M.)
	0	3.9 ± 0.1	0.39 ± 0.05	10.4 ± 1.2
	15	1.9 ± 0.1	0.20 ± 0.02	341.0 ± 23.0
	30	1.2 ± 0.1	0.11 ± 0.01	109.0 ± 13.0
	45	2.1 ± 0.1		
	60	2.3 ± 0.1	0.07 ± 0.01	26.6 ± 2.2
	75	2.6 ± 0.2		
	90	2.9 ± 0.2	(0.05 ± 0.01)	15.6 ± 1.8
	105	3.1 ± 0.2		
	120	3.4 ± 0.2	(0.05 ± 0.01)	12.1 ± 2.4
	135	3.7 ± 0.2		
	150	3.9 ± 0.2	0.06 ± 0.01	10.1 ± 2.1
	165	3.7 ± 0.2		
	180	3.9 ± 0.2	0.09 ± 0.02	9.0 ± 1.6
	195	3.9 ± 0.2		
	210	3.9 ± 0.2	0.11 ± 0.03	8.3 ± 1.3
MEAL				
	225	4.7 ± 0.2	0.25 ± 0.05	21.2 ± 0.5
	240	6.7 ± 0.3	0.76 ± 0.15	46.8 ± 7.1
	270	8.4 ± 0.3	1.72 ± 0.24	81.6 ± 12.7
	330	7.5 ± 0.3	2.58 ± 0.47	123.5 ± 14.0

TABLE 4.4

MEAN BLOOD GLUCOSE, PLASMA C-PEPTIDE AND INSULIN IN NORMAL SUBJECTS IN RESPONSE TO MEAL ALONE.

Equivalent times in hypoglycaemia study shown in brackets.

TIME (min)	BLOOD GLUCOSE (mmol/l) (mean ± S.E.M.)	PLASMA C-PEPTIDE (nmol/l) (mean ± S.E.M.)	PLASMA INSULIN (mU/l) (mean ± S.E.M.)
0	3.8 ± 0.1	0.35 ± 0.01	9.1 ± 0.6
MEAL			
15 (225)	4.4 ± 0.1	0.70 ± 0.04	29.0 ± 3.6
30 (240)	5.1 ± 0.2	1.76 ± 0.08	72.4 ± 5.2
60 (270)	3.6 ± 0.3	1.65 ± 0.18	51.0 ± 7.2
120 (330)	3.5 ± 0.2	1.16 ± 0.14	29.5 ± 4.5

glucose concentrations following the meal between the two studies was highly significant at 30, 60 and 120 min ($p < 0.001$).

Plasma C-peptide: The mean plasma C-peptide concentrations in the hypoglycaemia and control studies are shown in Table 4.3. In the hypoglycaemia study the mean plasma C-peptide fell rapidly after administration of insulin, from a fasting level of 0.39 ± 0.04 nmol/l (range 0.20 to 0.69 nmol/l) to the effective detection limit of the assay (0.06 nmol/l), 60 min after the injection of insulin (Fig. 4.5). It remained low throughout the period of blood glucose recovery and at 210 min after insulin was only 0.11 ± 0.03 nmol/l. C-peptide levels rose following the meal reaching 1.72 ± 0.24 nmol/l at 60 min and 2.58 ± 0.47 nmol/l at 120 min post-prandially (Fig. 4.6).

In the control study, mean plasma C-peptide rose from 0.35 ± 0.01 nmol/l to a peak of 1.76 ± 0.08 nmol/l at 30 min, falling to 1.16 ± 0.14 nmol/l at 120 min (Table 4.4). The differences in the mean plasma C-peptide concentrations between the two studies were significant ($p < 0.001$) at 15, 30 and 120 min after the meal.

Plasma insulin: The mean plasma insulin concentrations in the hypoglycaemia and control studies are shown in Table 4.3. In the hypoglycaemia study the expected rise in plasma after the injection of exogenous insulin was observed (Fig. 4.7), and was followed by an exponential fall. At the time of the meal the plasma insulin had returned to the fasting level. After ingestion of food, mean plasma insulin rose from 8.3 ± 1.3 mU/l to 81.6 ± 12.7 mU/l at 60 min and 123.5 ± 14 mU/l at 120 min.

In the control study, the mean plasma insulin rose from 9.1 ± 0.6 mU/l to 72.4 ± 0.5 mU/l at 30 min, falling to 51.0 ± 7.2 mU/l at 60 min and 29.5 ± 4.5 mU/l at 120 min (Table 4.4). The differences

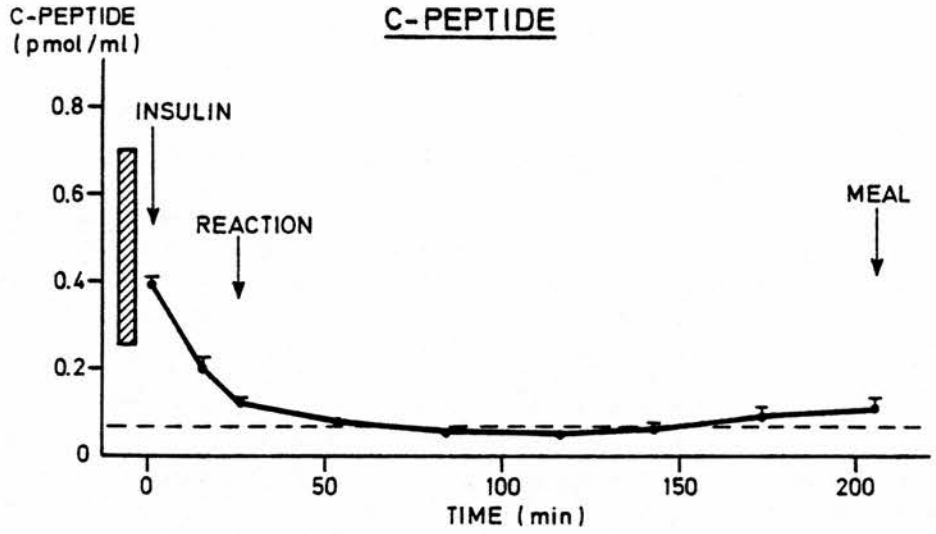

Fig. 4.5 Plasma C-peptide concentration (mean ± SEM) in response to hypoglycaemia (n = 11). The normal basal range in these normal subjects is shown (shaded bar) and the effective detection limit of the assay is indicated by the dotted line.

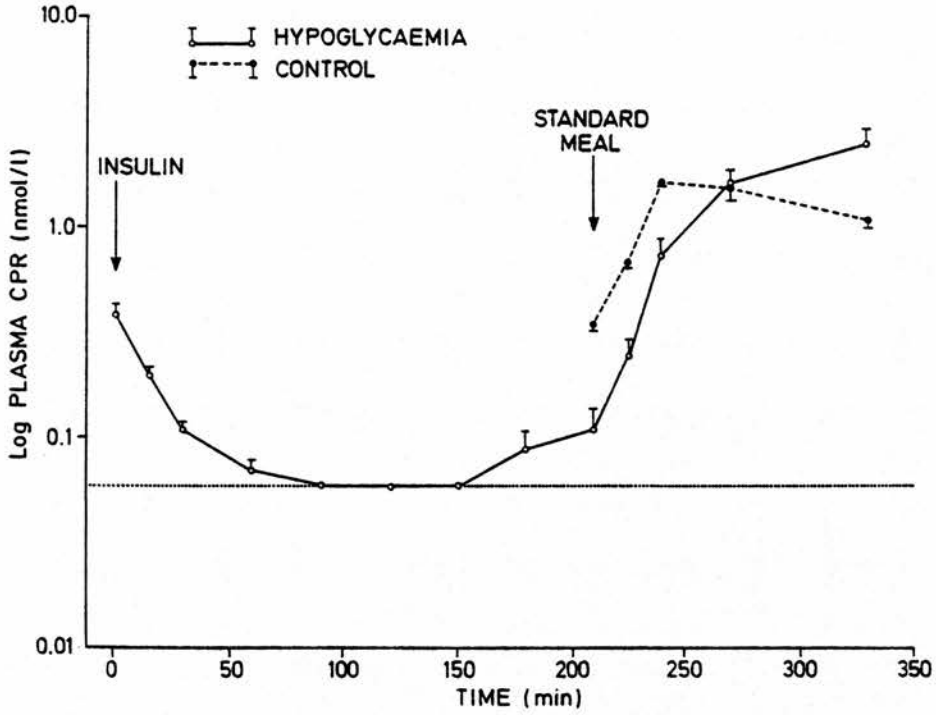

Fig. 4.6 Log plasma C-peptide concentration (mean ± SEM) in the hypoglycaemia and control studies (n = 9). CPR = C-peptide immunoreactivity. The effective detection limit of the assay is shown by a dotted line.

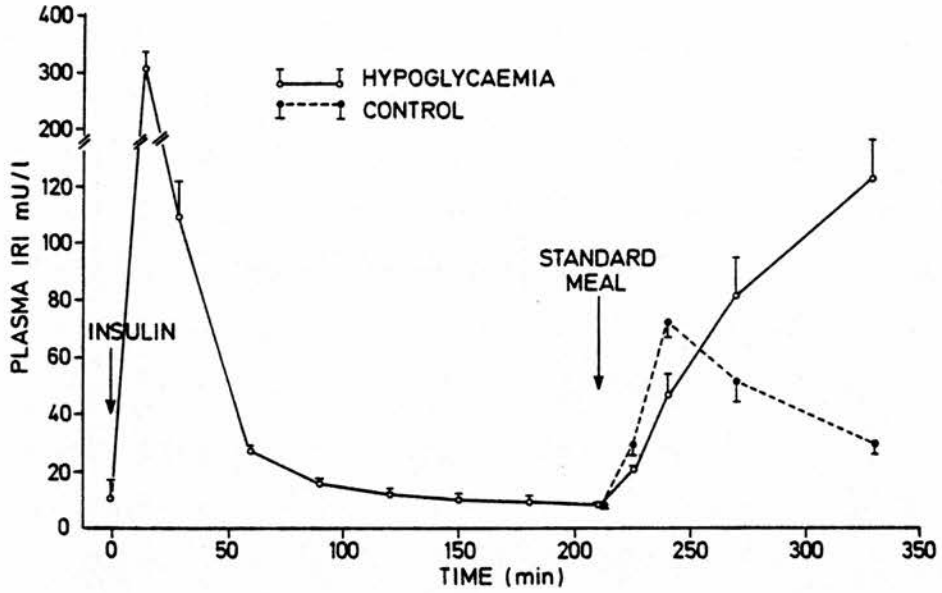

Fig. 4.7 Plasma insulin concentration (mean ± SEM) in the hypoglycaemia and control studies ($\bar{n}$ = 9). IRI = immunoreactive insulin.

in plasma insulin between the two studies were statistically significant ($p < 0.001$) at all four times of measurement after the meal.

DISCUSSION

The rapid fall of blood glucose following administration of insulin and the typical recovery pattern from hypoglycaemia were observed in all subjects, with normoglycaemia being restored by 150 minutes. The plasma insulin concentrations after the administration of exogenous insulin closely resemble those reported by Garber et al., (1976), and had fallen to within the fasting range prior to ingestion of the meal. The decline in the mean plasma insulin concentration during recovery from hypoglycaemia was not however comparable with the marked fall in plasma C-peptide concentration to undetectable levels, and is inconsistent with the short half-life of insulin in plasma. Appropriately low levels of plasma insulin were observed in three subjects with concentrations falling to less than 6.0 mU/l by 150 minutes after the administration of insulin, but it is difficult to explain the overall discrepancy between mean plasma insulin and C-peptide concentrations prior to the meal.

The prolonged suppression of plasma C-peptide concentration (and thus of endogenous insulin secretion) following insulin-induced hypoglycaemia is consistent with similar observations using insulin infusions (Horwitz et al., 1975b; Service et al., 1977). This prolonged suppression of insulin secretion may be a homeostatic mechanism to allow maintenance of a normal blood glucose concentration while glycogen stores are being replenished by increased hepatic gluconeogenesis. This possibility is supported by isotope infusion studies in man which have shown that hepatic glucose production from alanine is greatly increased two hours after the injection of insulin (Garber et al., 1976).

Impaired tolerance to oral glucose, ingested 40 to 50 minutes after the administration of insulin, was described both in normal and in insulin-dependent diabetic subjects (Somogyi, 1951), and was attributed to the secretion of hormones which antagonise the actions of insulin. The time of administration of the oral glucose after insulin would have coincided with the peak responses of the major counterregulatory hormones in response to hypoglycaemia, in particular with those of the catecholamines and glucagon (Chapter 1). These hormones would counteract the effect of insulin at this time after hypoglycaemia, and certainly could promote glucose intolerance at this stage of the metabolic recovery from hypoglycaemia. However, impairment of tolerance to oral glucose (100 G), administered 2½ hours after intravenous insulin, has also been demonstrated (Yalow et al., 1969) and was thought to correlate with the magnitude of the growth hormone response during the preceding hypoglycaemia. This impaired tolerance to oral glucose was associated with hyperinsulinism but there was no delay in the initial insulin secretory response to oral glucose when compared with control oral glucose tolerance tests in the same subjects without preceding hypoglycaemia. The impaired oral glucose tolerance following hypoglycaemia described by Yalow et al., (1969) is similar to the carbohydrate intolerance after ingestion of a mixed meal following hypoglycaemia in the present studies. (The growth hormone response to hypoglycaemia in the normal subjects in the present study is presented in Chapter 8 (Fig. 8.6). A peak concentration was achieved at 90 minutes after insulin administration, and the mean plasma growth hormone level had almost returned to basal levels by the time of ingestion of the meal).

In the present study the prolonged suppression of pancreatic beta cell secretion after injection of exogenous insulin was demonstrated

at 210 minutes by the persistence of a low concentration of plasma C-peptide, which had not regained the basal value despite restoration of normoglycaemia. It was pertinent therefore to examine the pancreatic beta cell response to the ingestion of food at this time interval after the administration of insulin to induce hypoglycaemia. Insulin secretion in response to a meal following hypoglycaemia was abnormal and was associated with impaired carbohydrate intolerance. In the present study an initial delay in the secretion of insulin after the meal was observed following hypoglycaemia, and must be responsible in part for the elevated post-prandial blood glucose levels. The hypersecretion of insulin observed 120 minutes after the meal may be a direct response to sustained hyperglycaemia.

The pattern of insulin secretion in these normal subjects exposed to preceding hypoglycaemia bears a marked similarity to the secretory response of insulin to oral glucose in patients with mild insulin-independent diabetes (Yalow and Berson, 1960; Seltzer et al., 1967; Bagdade et al., 1967). In mild diabetes, the initial secretion of insulin is delayed following both oral and intravenous glucose, but subsequent hyperinsulinaemia occurs in concert with prolonged hyperglycaemia. This excessive secretion of insulin is not observed in patients with diabetes of greater severity, despite higher blood glucose levels (Seltzer et al., 1967). One can speculate therefore that hypoglycaemia may temporarily affect pancreatic beta cell secretion in normal subjects via a mechanism similar to that present in patients with mild diabetes, although in the diabetic state it is likely that many other pathogenetic factors are involved.

Sustained stimulation by glucose produces a biphasic pattern of insulin secretion from the pancreatic beta cell <u>in vivo</u> (Cerasi and Luft, 1967; Porte and Pupo, 1969) and <u>in vitro</u> (Grodsky et al., 1967;

Curry et al., 1968). Phase 1 consists of a rapid surge of insulin secretion which reaches a peak value within a few minutes and then declines. In man (Cerasi and Luft, 1967) and in the rat (Curry et al., 1968) this is followed by a slower progressive rise in the rate of insulin secretion which is designated phase 2. The abnormal pattern of insulin secretion in mild diabetes is thought to result from an abnormality of phase 1 of the insulin secretory response (Cerasi and Luft, 1969; Grodsky, 1975), and it is possible that in the normal subjects exposed to preceding hypoglycaemia in this study, the abnormal insulin secretion in response to food principally implicates phase 1 of insulin secretion. This hypothesis is supported by perifusion studies of rat pancreatic islets, which demonstrate that the insulin secretory response to glucose is reduced markedly following prolonged exposure to a low basal concentration of glucose (Ashby and Shirling, 1980), which presumably induces a degree of intracellular glucopenia within the pancreatic islets. In this in vitro model, both phases of insulin secretion were affected, but the phase 1 response was impaired to a greater extent.

The present study may be of relevance to the interpretation of the impairment of diabetic control in patients with insulin-dependent diabetes, where rebound hyperglycaemia follows hypoglycaemia (Somogyi, 1959a; 1959b). Although some pancreatic beta cell reserve does exist in the early years of insulin-dependent diabetes (Binder and Faber, 1978), the majority of these diabetic subjects have insignificant endogenous insulin secretion. It seems unlikely therefore that the "Somogyi phenomenon" can be explained by suppression of beta cell secretion following insulin-induced hypoglycaemia, which has been demonstrated in subjects with normal endocrine pancreatic function.

The possible mechanisms underlying the abnormal secretion of insulin in normal subjects in response to a meal after hypoglycaemia will be examined in Chapter 5.

CHAPTER 5

THE MECHANISM OF THE ABNORMAL PANCREATIC BETA CELL RESPONSE TO FOOD FOLLOWING ACUTE HYPOGLYCAEMIA IN MAN

Chapter 5

The mechanism of the abnormal pancreatic beta cell response to food following acute hypoglycaemia in man

Introduction

Subjects and Methods:

1. Entero-insular axis: gastro-intestinal hormones
2. Effect of pancreatic beta cell glucopenia: glucose infusion
3. Effect of pancreatic beta cell cyclic AMP depletion: aminophylline infusion
4. Effect of catecholamine inhibition

Results:

1. G.I. hormones
2. Glucose infusion
3. Aminophylline infusion
4. Catecholamines

Discussion

In the previous chapter (Chapter 4) the abnormal function of the pancreatic beta cell in response to a meal following acute hypoglycaemia, was described in normal man. Following the ingestion of food, carbohydrate intolerance was associated with an abnormal pattern of insulin secretion, characterised by a delay in the early post-prandial rise of plasma insulin and an elevation two hours later. Possible mechanisms underlying this altered pattern of post-prandial insulin secretion following hypoglycaemia include:

(1) disordered function of the entero-insular axis following hypoglycaemia,

(2) intrinsic pancreatic beta cell dysfunction caused by glucopenia,

(3) pancreatic beta cell dysfunction caused by cyclic AMP depletion,

(4) inhibition of beta cell secretion by catecholamines released during hypoglycaemia, and

(5) inhibition of endogenous insulin secretion by insulin itself via feedback regulation.

In the hypoglycaemia study described in Chapter 4, plasma insulin levels had returned to within the normal basal range prior to ingestion of the meal, therefore the possible existence of a short-loop feedback mechanism on endogenous insulin secretion was not examined. The relative contributions of the other possible mechanisms were studied by the investigations described in this chapter.

SUBJECTS AND METHODS

The basic protocol has been described in Chapter 4. A hypoglycaemia study and a control (or fasting) study was performed in each subject,

and the following possible mechanisms were investigated, using modifications to the protocol as described.

(1) Entero-insular axis: gastro-intestinal hormones. Serial blood samples were taken during the hypoglycaemia and control studies in six normal, non-obese male subjects who participated in the study described in Chapter 4 (subjects no. 6 - 11, Table 4.1), age range 21 - 29 years. Radioimmunoassays were made (Dr. S.R. Bloom's laboratory) of plasma enteroglucagon (Thompson and Bloom, 1976), motilin (Bloom et al., 1976), neurotensin (Blackburn and Bloom, 1979), gastrin (Russell et al., 1976), gastric inhibitory peptide (Sarson et al., 1980) and pancreatic polypeptide (Adrian et al., 1976). Cross-reactivity of the antisera raised to the various peptides assayed was less than 0.5 per cent. Antiserum R59, which showed a high degree of cross-reactivity with ileal extracts, and fully detected gravimetrically-determined glicentin, was used to measure total plasma glucagon. Enteroglucagon concentration was derived by subtraction of the pancreatic glucagon concentration measured by the pancreatic specific antiserum RCS5 (Bloom, 1974), from total plasma glucagon.

(2) Effect of pancreatic beta cell glucopenia: glucose infusion: A possible effect of hypoglycaemia on pancreatic beta cell function was evaluated by the administration of intravenous glucose to four other normal subjects (3 male, 1 female; Table 5.4) using a modification of the basic protocol. At 150 minutes after the injection of insulin, dextrose (0.5 g/kg body weight) was infused intravenously over three minutes in a total volume of 250 ml (Soeldner, 1971) either following hypoglycaemia or after an equivalent period of fasting. Serial measurements were made of blood glucose, plasma insulin and plasma C-peptide (as described in Chapter 4). After the i.v. glucose load,

blood glucose was determined at 10 minute intervals for 60 minutes prior to the ingestion of the standard meal. In the control (fasting) study an identical infusion of dextrose was given at an equivalent time prior to the standard meal, and similar measurements were made.

(3) Effect of pancreatic beta cell depletion of cAMP: aminophylline infusion: A possible effect of depletion of cyclic AMP within the pancreatic beta cell in the production of the abnormal pattern of insulin secretion following hypoglycaemia, was investigated in two of the normal subjects. These two male subjects (aged 21 and 23 years) were given aminophylline after hypoglycaemia in an attempt to inhibit phosphodiesterase activity within the pancreatic beta cells, to replenish the intracellular concentration of cyclic AMP and enhance a normal pattern of insulin secretion in response to food. Aminophylline, 250 mg i.v., was administered as a bolus injection 150 minutes after insulin, which was followed by an infusion of aminophylline, 4 mg/min, for 60 minutes prior to the meal. Serial measurements were made of blood glucose, plasma insulin and plasma C-peptide concentrations.

(4) Effect of catecholamine inhibition: The effect of hypoglycaemia on insulin secretion in response to a meal was examined in two male subjects (aged 22 and 44 years) with traumatic transection of the cervical spinal cord above the sympathetic outflow. This produces a pre-ganglionic sympathectomy, and further studies utilising this human model of adrenergic denervation will be discussed in detail in Chapters 6 - 8. Serial measurements were made of blood glucose, plasma insulin and plasma C-peptide concentrations. Plasma noradrenaline was assayed (Henry et al., 1975) in the basal state and 45 minutes after the administration of insulin.

Statistics: Results are expressed as mean ± 1 S.E.M. Statistical significance was assessed using Student's t test for paired data. The rate of glucose disappearance, K_{bg}, following intravenous infusion of glucose was calculated using the method described by Duncan (1956).

RESULTS

(1) G.I. Hormones. The mean blood glucose, plasma insulin and C-peptide concentrations of the group of six normal subjects were determined in both the hypoglycaemia and control studies. The serial changes in these parameters were identical to the patterns observed in the larger group of 11 normal subjects (Chapter 4) and are shown in Fig. 5.1. The changes in plasma pancreatic glucagon levels in this group of six subjects are illustrated in Fig. 5.2. Plasma pancreatic glucagon rose from a mean basal value of 8.2 ± 1.2 pmol/l to a peak of 36.6 ± 9.5 pmol/l at 60 min after the administration of insulin ($p < 0.001$), returning towards basal values prior to the meal. Although there was a tendency for plasma pancreatic glucagon to rise following the meal in the control study, there was no significant difference between the control and hypoglycaemia studies in the post-prandial response of pancreatic glucagon.

The mean values of the individual gastro-intestinal hormones in the same six subjects are shown during the hypoglycaemia study (Table 5.1) and control study (Table 5.2). There was a pronounced rise in plasma pancreatic polypeptide in response to hypoglycaemia (Fig. 5.3) with a peak value at 60 min after insulin, and a further smaller rise in response to food, which did not differ from the

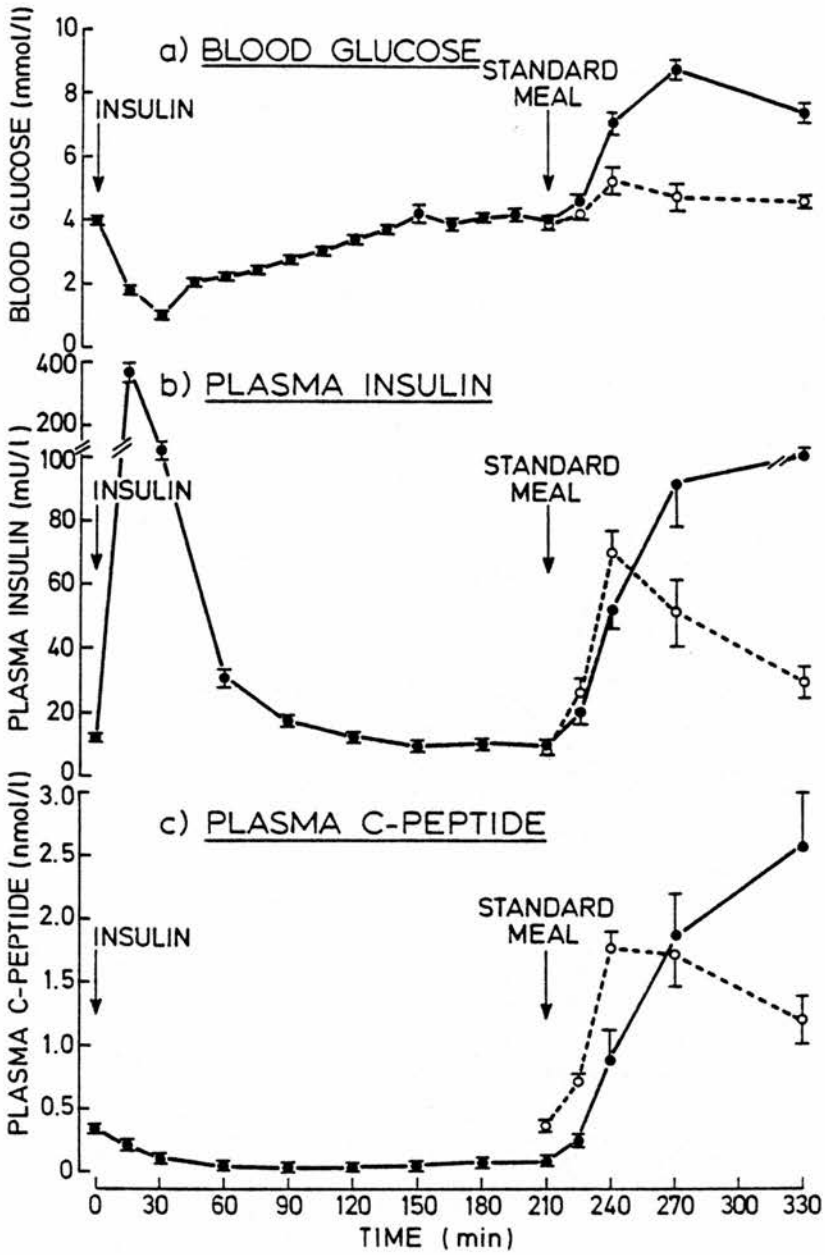

Fig. 5.1 Blood glucose, plasma insulin and C-peptide concentrations (mean ± SEM) in six normal subjects, in response to hypoglycaemia followed by a standard meal (hypoglycaemia study ●—●), and in response to a standard meal without preceding hypoglycaemia (control study ○--○).

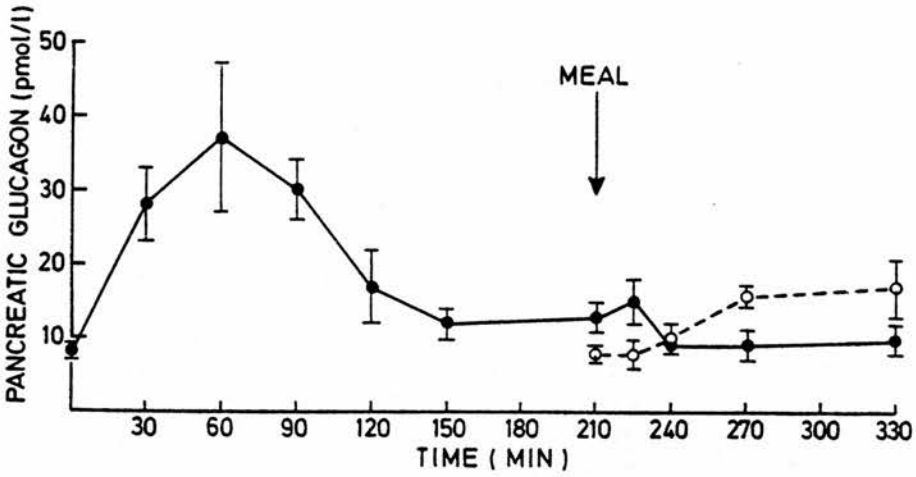

Fig. 5.2 Plasma pancreatic glucagon concentrations (mean ± SEM) in response to hypoglycaemia (insulin administered at time 0) followed by a standard meal (●——●) and in response to a standard meal without preceding hypoglycaemia (○– –○). (n = 6).

TABLE 5.1

G.I. HORMONES DURING HYPOGLYCAEMIA STUDY

Mean ± S.E.M. (pmol/l)

	TIME (min)	PANCREATIC POLYPEPTIDE	GASTRIN	GASTRIC INHIBITORY PEPTIDE	ENTEROGLUCAGON	NEUROTENSIN	MOTILIN
	0	24 ± 6	3.0 ± 0.9	5 ± 1	24 ± 5	63 ± 11	55 ± 11
	30	508 ± 103	2.8 ± 0.5	6 ± 1	20 ± 6	66 ± 20	48 ± 13
	60	935 ± 120	3.2 ± 0.8	7 ± 2	20 ± 5	67 ± 18	59 ± 13
	90	776 ± 129	3.7 ± 0.9	11 ± 2	16 ± 3	57 ± 23	67 ± 14
	120	465 ± 157	3.8 ± 1.2	10 ± 2	22 ± 6	77 ± 29	70 ± 15
	150	235 ± 48	4.5 ± 1.0	8 ± 3	29 ± 9	40 ± 7	65 ± 14
	210	124 ± 11	5.0 ± 0.9	6 ± 1	25 ± 5	66 ± 30	83 ± 15
MEAL							
	225	515 ± 139	6.5 ± 0.8	16 ± 5	37 ± 12	71 ± 33	84 ± 11
	240	480 ± 114	6.5 ± 1.2	39 ± 16	29 ± 7	63 ± 21	67 ± 14
	270	306 ± 110	7.3 ± 1.6	43 ± 15	48 ± 12	77 ± 28	52 ± 13
	330	258 ± 96	9.2 ± 1.9	42 ± 11	46 ± 8	88 ± 21	42 ± 12

TABLE 5.2

G.I. HORMONES DURING CONTROL (FASTING) STUDY

Mean ± S.E.M. (pmol/l)

Comparable times to hypoglycaemia study shown in brackets

	TIME (min)	PANCREATIC POLYPEPTIDE	GASTRIN	GASTRIC INHIBITORY PEPTIDE	ENTEROGLUCAGON	NEUROTENSIN	MOTILIN
	0	26 ± 4	3.8 ± 0.9	7 ± 2	25 ± 4	75 ± 20	54 ± 10
MEAL							
	15 (225)	525 ± 159	4.8 ± 0.9	17 ± 7	28 ± 5	73 ± 18	103 ± 10
	30 (240)	521 ± 111	8.0 ± 0.9	47 ± 17	44 ± 7	70 ± 20	75 ± 18
	60 (270)	325 ± 104	8.5 ± 1.8	54 ± 19	46 ± 6	70 ± 13	53 ± 12
	120 (330)	322 ± 89	7.5 ± 1.7	58 ± 16	52 ± 6	56 ± 13	47 ± 11

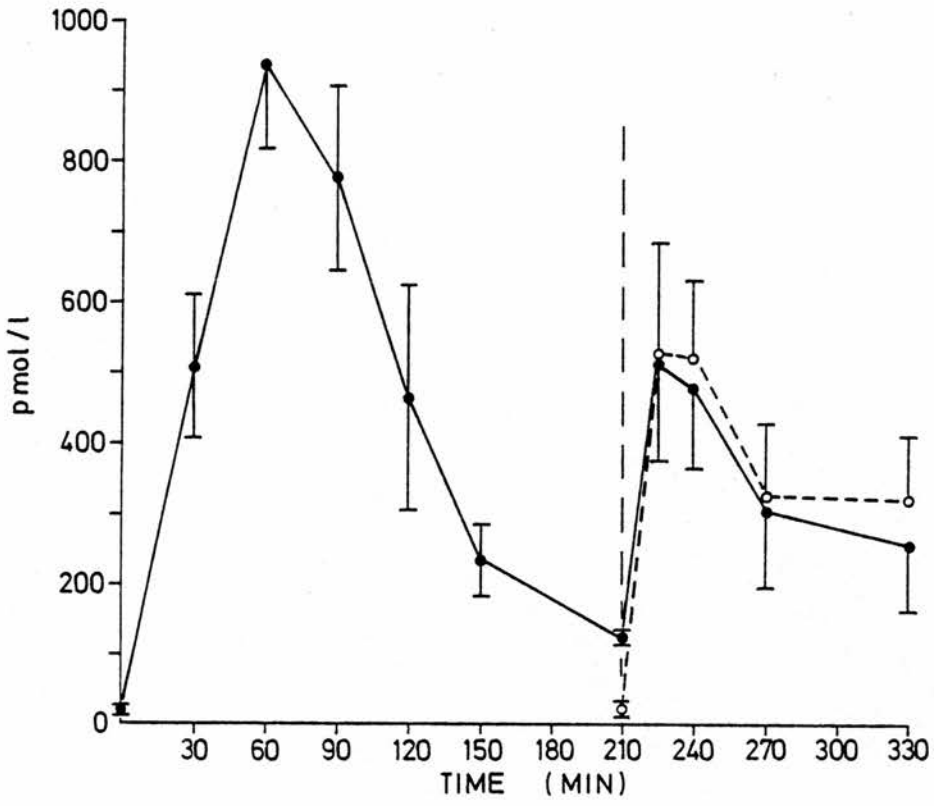

Fig. 5.3 Plasma pancreatic polypeptide concentrations (mean ± SEM) in response to hypoglycaemia (insulin administered at time 0) followed by a standard meal at 210 min (●—●), and in response to a standard meal without preceding hypoglycaemia (○--○). (n = 6).

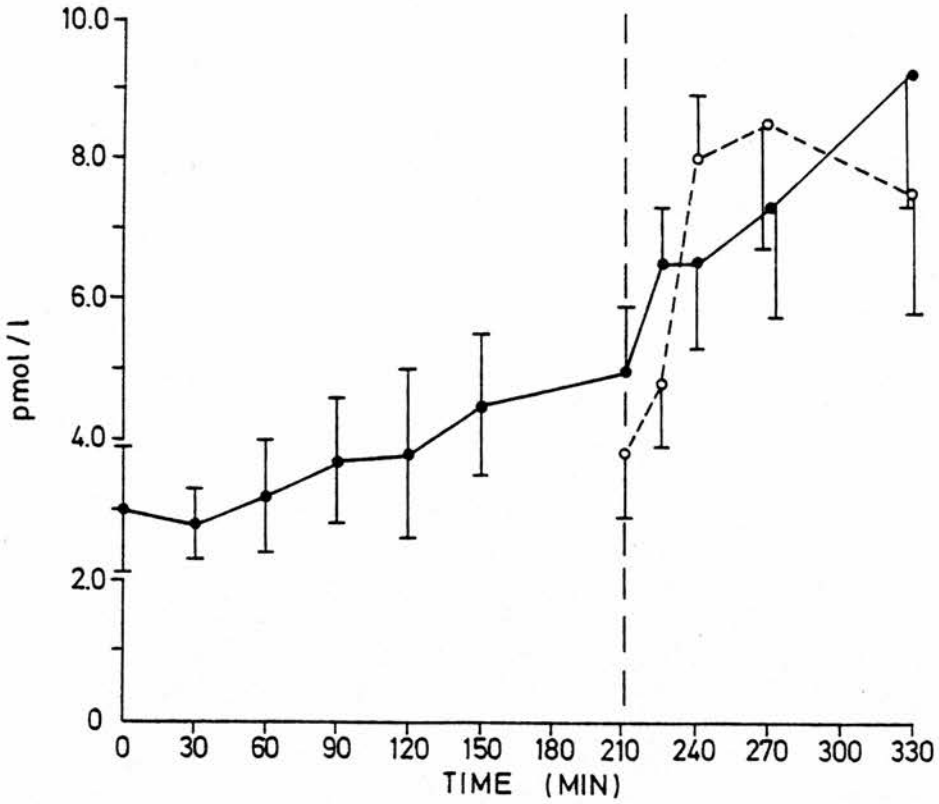

Fig. 5.4 Plasma gastrin concentrations (mean ± SEM) in response to hypoglycaemia (insulin administered at time 0) followed by a standard meal at 210 min (●—●), and in response to a standard meal without preceding hypoglycaemia (○- -○). (n = 6).

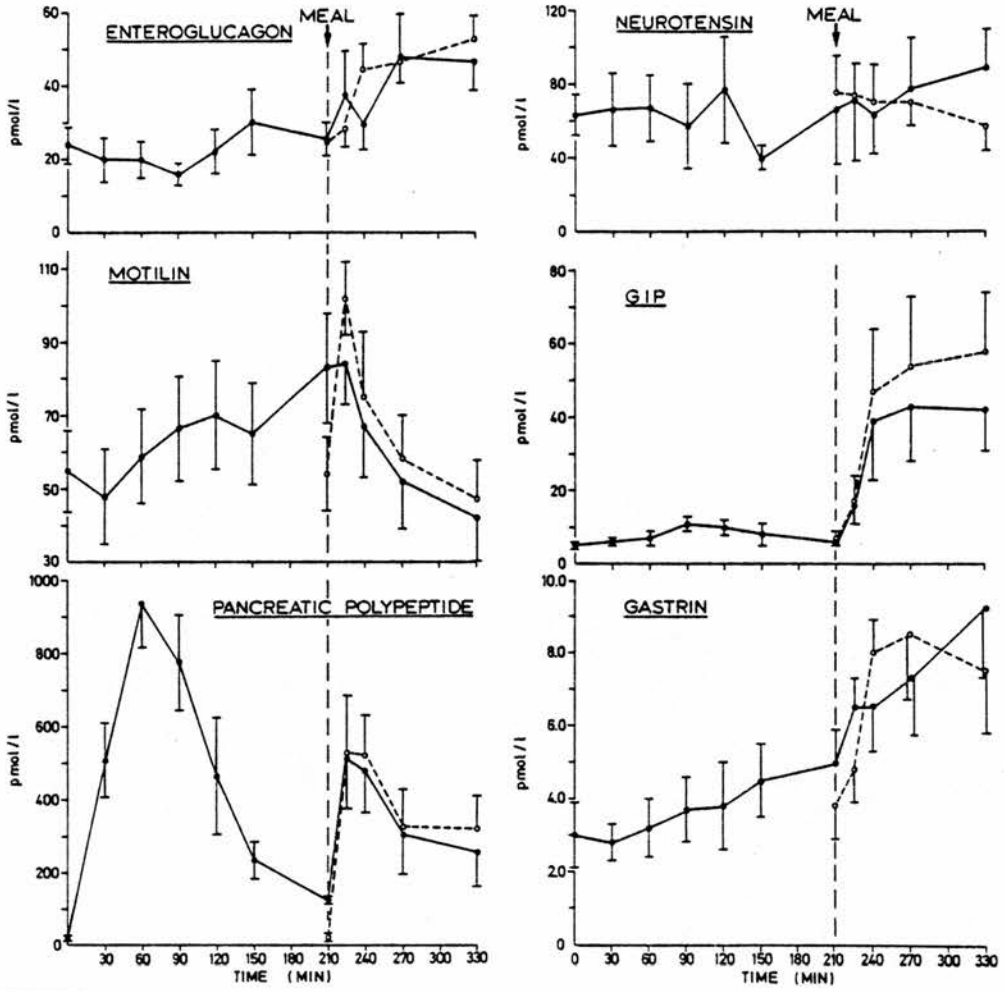

Fig. 5.5 Plasma concentrations (mean ± SEM) of gastro-intestinal hormones in six normal subjects in the hypoglycaemia (●——●) and control (○--○) studies. Insulin was administered at time 0, and the meal at 210 min.

control study. A gradual but statistically insignificant rise during hypoglycaemia was observed in plasma gastrin (Fig. 5.4) (and motilin) levels, but the other G.I. hormones remained unchanged during hypoglycaemia (Fig. 5.5). There was no difference in the pattern of G.I. hormone secretion after the meal when the hypoglycaemia and control studies were compared.

(2) Glucose infusion. The changes in blood glucose, plasma insulin and C-peptide in the four subjects who received i.v. glucose 60 minutes before the meal are shown in Table 5.3 and illustrated in Fig. 5.6. Following the i.v. infusion of glucose, the peak blood glucose level was comparable in both the hypoglycaemia and control studies, but the mean level then fell to a significantly lower level in the control study both at 50 and at 60 minutes after the injection of glucose ($p < 0.05$). In all four subjects the rate of glucose disappearance, K_{bg}, (Duncan, 1956) was slower during the hypoglycaemia study (Table 5.4). The mean K_{bg} was lower in the hypoglycaemia study, but did not achieve statistical significance when compared with the mean K_{bg} in the control study. Following the meal, the mean blood glucose levels were significantly higher after preceding hypoglycaemia than mean blood glucose in the control study at all times of measurement ($p < 0.001$). However, both the incremental rise from the different pre-prandial mean blood glucose levels and the subsequent pattern of glucose tolerance were similar in both the hypoglycaemia and control studies. This differs markedly from the post-prandial blood glucose levels observed after hypoglycaemia alone (Fig. 5.6: shaded area).

Mean plasma insulin and C-peptide concentrations both rose in response to the infusion of glucose, and in individual subjects the increments of plasma insulin and of C-peptide were smaller following hypoglycaemia. The mean increment of plasma insulin in response to

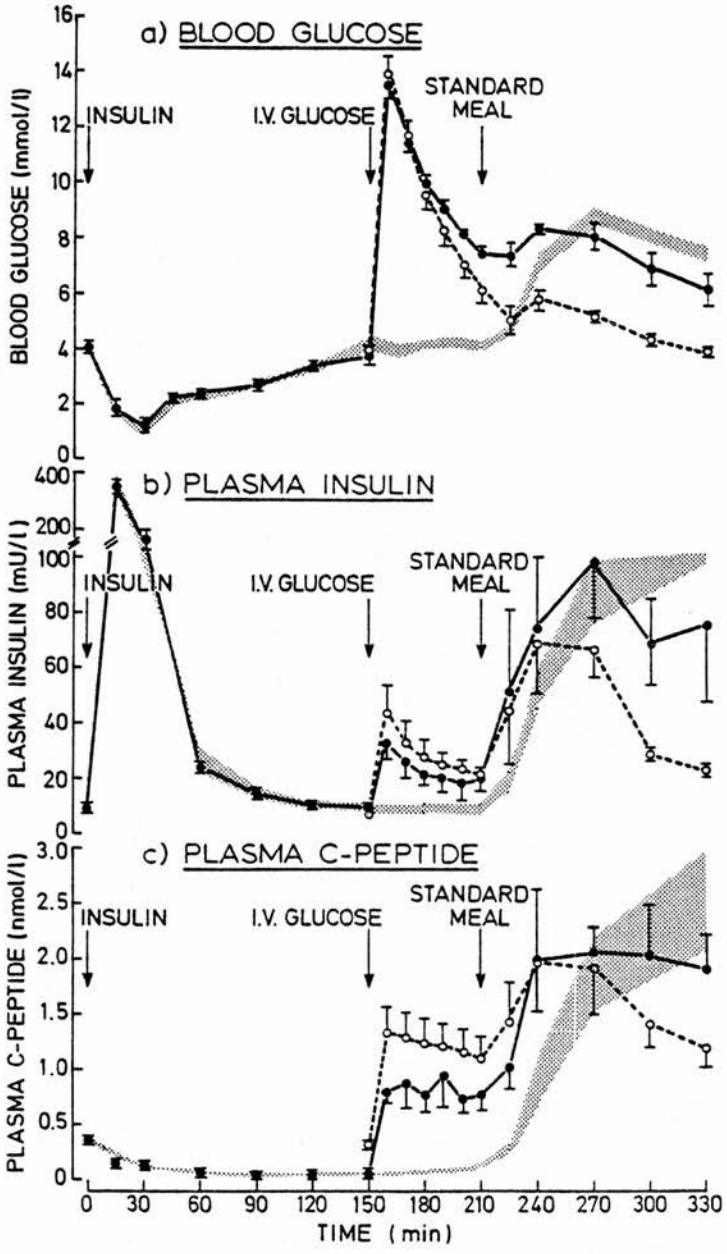

Fig. 5.6 Intravenous glucose was administered 150 min after insulin injection to four normal subjects, prior to a standard meal (hypoglycaemia study ●—●) and after an equivalent period of fasting (control study ○--○). Blood glucose, plasma insulin and C-peptide concentrations (mean ± SEM) are shown. The shaded area represents the mean ± SEM of normal subjects (n = 9, Table 4.3) following insulin administration without i.v. glucose.

TABLE 5.3

BLOOD GLUCOSE, PLASMA INSULIN AND C-PEPTIDE CONCENTRATIONS(Mean ± S.E.M.) IN HYPOGLYCAEMIA AND CONTROL STUDIES

Effect of I.V. glucose at 150 min after insulin on response to subsequent meal

		BLOOD GLUCOSE (mmol/l)		PLASMA INSULIN (mU/l)		PLASMA C-PEPTIDE (nmol/l)	
	TIME (min)	HYPOGLYCAEMIA STUDY	CONTROL STUDY	HYPOGLYCAEMIA STUDY	CONTROL STUDY	HYPOGLYCAEMIA STUDY	CONTROL STUDY
	150	3.8 ± 0.3	4.0 ± 0.1	10 ± 2	7 ± 2	0.05 ± 0.02	0.32 ± 0.03
	160	13.5 ± 0.6	13.9 ± 0.6	33 ± 6	44 ± 10	0.79 ± 0.12	1.32 ± 0.23
	170	11.5 ± 0.3	11.7 ± 0.6	26 ± 6	33 ± 8	0.86 ± 0.23	1.28 ± 0.23
	180	10.0 ± 0.3	9.5 ± 0.5	22 ± 4	28 ± 6	0.75 ± 0.15	1.22 ± 0.23
	190	9.1 ± 0.3	8.2 ± 0.5	21 ± 5	26 ± 4	0.93 ± 0.30	1.20 ± 0.20
	200	8.2 ± 0.1	7.1 ± 0.4	19 ± 6	23 ± 3	0.72 ± 0.14	1.15 ± 0.21
	210	7.5 ± 0.3	6.2 ± 0.5	21 ± 5	21 ± 3	0.76 ± 0.15	1.08 ± 0.20
MEAL							
	225	7.4 ± 0.5	5.1 ± 0.5	52 ± 29	44 ± 19	1.02 ± 0.20	1.41 ± 0.36
	240	8.3 ± 0.2	5.8 ± 0.4	75 ± 26	69 ± 18	1.98 ± 0.67	1.95 ± 0.43
	270	8.1 ± 0.5	5.2 ± 0.3	98 ± 20	67 ± 10	2.05 ± 0.27	1.80 ± 0.33
	300	6.9 ± 0.6	4.4 ± 0.2	70 ± 16	29 ± 3	2.00 ± 0.47	1.40 ± 0.22
	330	6.2 ± 0.6	3.9 ± 0.2	76 ± 28	23 ± 1	1.90 ± 0.30	1.18 ± 0.20

TABLE 5.4

RATE OF GLUCOSE DISAPPEARANCE (K_{bg}) FOLLOWING I.V. GLUCOSE

SUBJECT	AGE & SEX	K_{bg} HYPOGLYCAEMIA STUDY	K_{bg} CONTROL STUDY
1	22 M	1.089	1.460
2	21 M	1.200	1.907
3	23 M	1.489	1.547
4	23 F	1.245	1.596
	MEAN	1.256	1.628
	S.E.M.	0.084	0.097

this glucose infusion was 23 ± 7 mU/l in the hypoglycaemia study, compared to 37 ± 12 mU/l in the control study. This difference did not achieve statistical significance in this small group of subjects. In response to the meal (preceded by parenteral glucose) the patterns of insulin secretion were similar in the hypoglycaemia and the control studies, but significantly higher mean values of plasma insulin and C-peptide were observed in the subjects following hypoglycaemia at 90 and at 120 minutes after the meal ($p < 0.001$). Following hypoglycaemia plus the glucose infusion the rises in plasma insulin and C-peptide were more rapid during the first 15 minutes after the meal in comparison with the equivalent rises after hypoglycaemia alone (Fig. 5.6).

(3) Aminophylline infusion. The effect of an infusion of aminophylline prior to the meal on the individual blood glucose, plasma insulin and C-peptide levels of two normal subjects is shown in Fig. 5.7. There was no significant effect on the post-prandial pattern of insulin secretion after hypoglycaemia, and glucose intolerance after the meal was observed in both subjects. The shaded area represents the mean ± one standard deviation of the group of six normal subjects.

(4) Catecholamines. The pattern of blood glucose, plasma insulin and C-peptide observed in two subjects with pre-ganglionic sympathectomy, in response to the meal following hypoglycaemia, are shown in Fig. 5.8. Post-prandial hyperglycaemia was again present, and was accompanied by a delayed hypersecretion of insulin and C-peptide. The pattern in these two subjects with adrenergic denervation was similar to that observed in normal subjects after hypoglycaemia. The plasma noradrenaline concentrations in the two tetraplegic subjects did not increase significantly in response to hypoglycaemia. The basal values in each subject were both less than 25 ng/l, and at 45 minutes

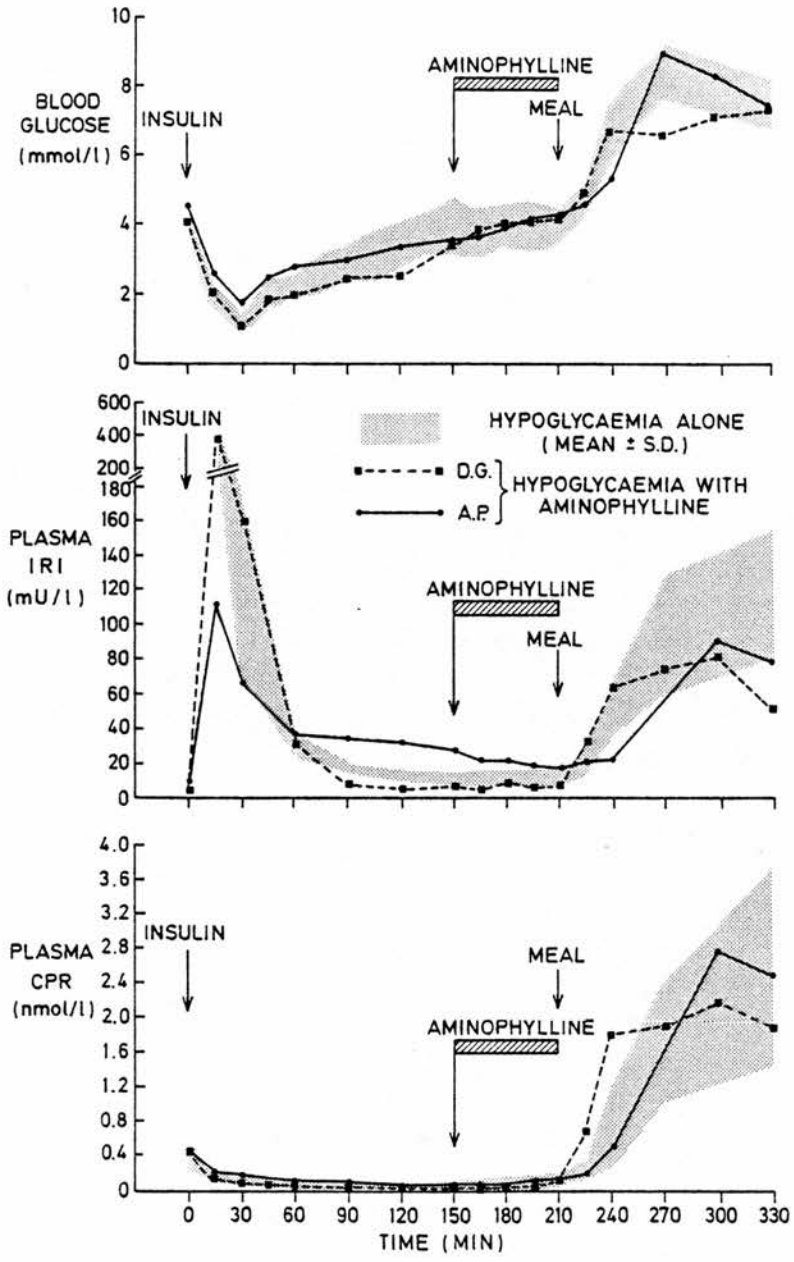

Fig. 5.7 Intravenous aminophylline was administered 150 min after insulin injection to two normal subjects, and the infusion of aminophylline was continued for 60 min prior to the standard meal. Blood glucose, plasma insulin (IRI) and C-peptide (CPR) concentrations of each subject are shown. The shaded areas represents the mean ± SD of normal subjects (n = 9) during the hypoglycaemia study without aminophylline administration.

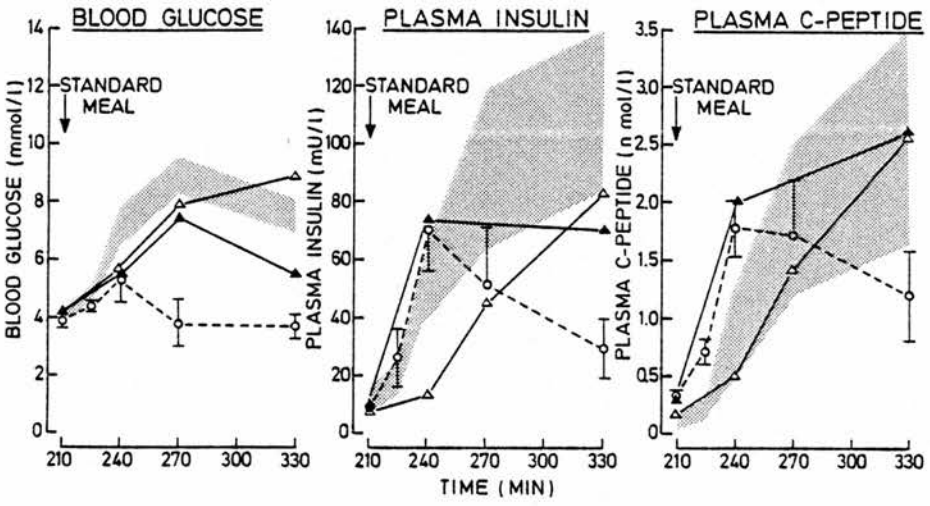

Fig. 5.8 Individual blood glucose, plasma insulin and C-peptide concentrations in two subjects with a pre-ganglionic sympathectomy, in response to a standard meal following hypoglycaemia. (Time represented from 210 min after insulin injection). The shaded area represents the response (mean ± 2SEM) in normal subjects following hypoglycaemia in response to the standard meal (n = 9). The response to a meal after fasting (mean ± SEM) in the same normal subjects is also shown (⊖–⊖).

after insulin were less than 25 and 61 ng/l respectively. The basal values in these tetraplegic subjects were much lower than those observed in normal subjects in whom there was also a marked response to hypoglycaemia (Chapter 6).

DISCUSSION

The investigation of acute hypoglycaemia in normal human subjects has demonstrated that the intravenous administration of short-acting insulin has a profound effect on subsequent insulin secretion by the pancreatic beta cell. This suppression of endogenous insulin secretion persists for a considerable period (at least three hours in the present studies) after the induction of hypoglycaemia. Impaired tolerance to oral glucose during the recovery from hypoglycaemia (Somogyi, 1951; Yalow et al., 1969) has been shown to occur also after ingestion of a mixed meal (Chapter 4). The present studies of the possible mechanisms underlying the abnormal secretion of insulin after hypoglycaemia, suggest that this is not caused by a reduced secretion of gastro-intestinal hormones in response to a meal. The impaired tolerance to parenteral glucose following hypoglycaemia also indicates that the underlying mechanism does not involve an abnormality of the entero-insular axis.

Following hypoglycaemia, the diminished insulin secretory response to intravenous glucose suggests that pancreatic beta cell function may be affected directly by the preceding hypoglycaemia. The early subnormal secretion of insulin after the meal, the later hypersecretion of insulin and the degree of post-prandial glucose intolerance were all less marked after the intravenous glucose load. Thus the impaired capacity of the pancreatic beta cell following hypoglycaemia to respond

to a meal, appeared to be reversed partially by a preceding intravenous infusion of glucose, which suggests that glucopenia of the pancreatic beta cells may be a causal factor.

Intracellular cyclic AMP has been implicated in the process of insulin secretion (Sharp, 1979) and intracellular depletion of thisnucleotide might explain the abnormal secretory pattern of insulin following hypoglycaemia. The administration of a phosphodiesterase inhibitor, aminophylline, to decrease the intracellular degradation of cyclic AMP, has been reported to improve the deficient insulin secretory response to intravenous glucose in a group of patients with "pre-diabetes" (Cerasi and Luft, 1969). These "pre-diabetic" subjects had a diminished insulin response to intravenous glucose compared with that of normal subjects. The dose of aminophylline used by Cerasi and Luft (1969) is comparable with the present study. They administered 200 mg intravenously as a priming dose, followed by 200 mg for 60 minutes as a continuous infusion prior to the performance of an intravenous glucose tolerance test. In the study by Cerasi and Luft (1969), aminophylline alone had no effect on the plasma insulin concentration, but improved the initial response of insulin to intravenous glucose in these "pre-diabetic" subjects. It was concluded that an increase in the intracellular concentration of cyclic AMP, promoted by aminophylline, restored phase 1 of insulin secretion, while phase 2 was not influenced. However, aminophylline did not enhance insulin secretion in normal, non-diabetic subjects (Cerasi and Luft, 1969), and in the present study had no effect on the defective post-prandial pattern of insulin secretion following hypoglycaemia. This suggests that the prolonged disturbance of pancreatic beta cell function after hypoglycaemia is not mediated by depletion of intracellular cyclic AMP in normal subjects.

Plasma catecholamines, which inhibit insulin secretion and rise markedly during hypoglycaemia (Garber et al., 1976), could also cause suppression of pancreatic beta cell function. The pattern of insulin secretion in response to a meal after preceding hypoglycaemia is similar to that observed during and after the intravenous infusion of adrenaline, whereby insulin secretion is suppressed until the infusion of adrenaline is discontinued (Robertson and Porte, 1973; Turner et al., 1977). Insulin secretion is delayed initially during the recovery period after the adrenaline infusion is discontinued, but hypersecretion of insulin occurs subsequently. The normal rise of plasma catecholamines in response to hypoglycaemia does not occur in tetraplegic subjects, and has been demonstrated by measurement of plasma noradrenaline (Palmer et al., 1976) and plasma adrenaline (Mathias et al., 1979). The plasma noradrenaline concentrations did not rise following hypoglycaemia in the two tetraplegic subjects in the present study, and the post-prandial pattern of insulin secretion was similar to that observed in normal subjects following hypoglycaemia. This suggests that an adrenergic mechanism is not responsible for the abnormal pancreatic beta cell function after hypoglycaemia.

The possibility that insulin directly inhibits its own secretion has been studied by other investigators by maintaining normoglycaemia with a glucose infusion during the administration of insulin. The partial suppression of plasma C-peptide without the production of hypoglycaemia was interpreted as evidence for the existence of a direct negative feedback mechanism (Liljenquist et al., 1978; Service et al., 1978), but was not confirmed by Shima et al., (1977). However, in the present study, the mean plasma insulin concentration had returned to within the normal basal range prior to the ingestion

of the meal. While a feedback mechanism by insulin itself is unlikely to explain the abnormal post-prandial secretion of insulin after acute hypoglycaemia, it is not possible to prove that the initial high plasma insulin concentration does not influence the pancreatic beta cells for several hours.

In summary, the present investigations suggest that the abnormal function of the pancreatic beta cell following acute hypoglycaemia in normal man is mediated in part by intracellular glucopenia and not by an abnormality of the entero-insular axis, intracellular cyclic AMP depletion of the beta cell, or an inhibition of insulin secretion by circulating catecholamines.

CHAPTER 6

MANIFESTATIONS OF ACUTE HYPOGLYCAEMIA IN SUBJECTS WITH PRE-GANGLIONIC SYMPATHECTOMY WITH AND WITHOUT CHOLINERGIC BLOCKADE

Chapter 6

Manifestations of acute hypoglycaemia in subjects with pre-ganglionic sympathectomy with and without cholinergic blockade

Introduction

Subjects and Methods: Protocol

Results: Clinical manifestations of hypoglycaemia
- Subjective symptoms
- Haemodynamic changes
- Haematocrit
- Sweating
- Plasma noradrenaline

Discussion

Few pathological conditions in man produce a degree of autonomic denervation that either can be considered to be complete or can be quantified with accuracy. The effect of autonomic denervation on metabolic processes has been studied in patients with autonomic neuropathy of varying causes, such as diabetes mellitus or the Shy-Drager syndrome, but in all of these conditions the autonomic neuropathy is patchy and incomplete, and may involve either the sympathetic or the parasympathetic division to varying degrees. Traumatic transection of the cervical spinal cord above the sympathetic outflow (T1 - L2) produces a total pre-ganglionic sympathectomy, and provides a valuable method to evaluate adrenergic mechanisms *in vivo* in response to hypoglycaemia in man. The administration of atropine to these tetraplegic subjects produces a significant degree of cholinergic blockade, and permits examination of the contribution of adrenergic and cholinergic neural activity to the homeostatic recovery from hypoglycaemia in man. The experimental model provided by the tetraplegic subject is represented diagrammatically in Fig. 6.1. The effects of insulin-induced hypoglycaemia were studied either without atropine (adrenergic denervation) or with atropine (combined adrenergic denervation and cholinergic blockade).

SUBJECTS AND METHODS

In the studies described in Chapters 6 - 8, three groups of subjects were investigated.

Group A: NORMAL subjects. The 11 normal healthy subjects (9 male, 2 female) described previously in Chapters 4 and 5, were used as a control group.

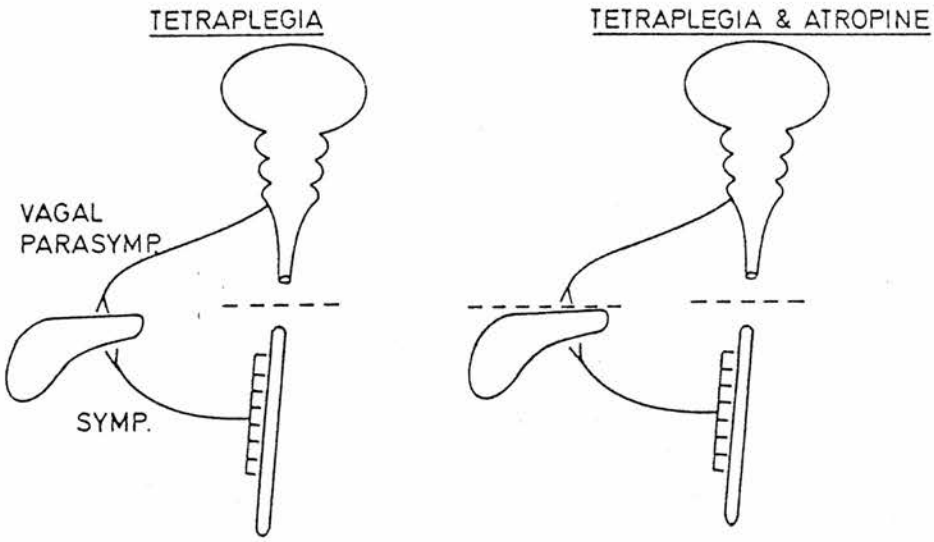

Fig. 6.1 Diagrammatic representation of the experimental model in tetraplegia. The parasympathetic and sympathetic innervation of an organ (e.g. pancreas, liver etc.) is shown. The dotted lines represent the sites of denervation of each division of the autonomic nervous system. The tetraplegic subject (left) has a pre-ganglionic sympathectomy producing adrenergic denervation alone. The administration of atropine (right) produces pharmacological blockade of cholinergic activity in addition to adrenergic denervation.

Group B: TETRAPLEGIC subjects. Six non-obese male subjects, with complete post-traumatic transection of the cervical spinal cord above C7 of not less than six months duration (range 6 months - 19 years) were studied. The age range was 21 - 44 years; clinical details of both tetraplegic groups (groups B and C) are shown in Table 6.1.

Group C: TETRAPLEGIC subjects given ATROPINE. Six non-obese male tetraplegic subjects (two from group B and four others) with similar post-traumatic transection of the spinal cord (duration 4 months - 5 years), age range 19 - 28 years, were studied.

None of the tetraplegic subjects had a family history of diabetes mellitus and all were otherwise well at the time of study. They were taking no medications at the time of each investigation.

Protocol: All subjects were studied supine after an overnight fast, and blood samples were taken via a teflon cannula situated in an arm vein. Basal blood samples were taken for 30 minutes before the administration of crystalline beef insulin, 0.15 units/kg body weight, which was given as a bolus by intravenous injection. This dose of insulin was used for each subject in all three groups. The tetraplegic subjects in group C were given atropine, 15 µg/kg body weight, which was administered 30 minutes before the insulin. The effectiveness of the cholinergic blockade was assessed by serial measurements of pulse rate, which was recorded at two minute intervals along with the blood pressure. Cholinergic blockade was maintained by a repeat injection of atropine if the pulse rate began to fall significantly.

Serial blood samples were taken at intervals. Specific assays of substrate and hormone concentrations are described principally in

TABLE 6.1

CLINICAL DETAILS OF TWO GROUPS OF TETRAPLEGIC SUBJECTS

GROUP B: Tetraplegic subjects without atropine

SUBJECT	AGE (years)	DURATION OF CERVICAL TRANSECTION	LEVEL OF TRANSECTION	PERCENTAGE IDEAL BODY WEIGHT
I.J.	21	5 years	C4/5	90
P.F.	44	13 years	C5/6	92
A.B.	23	6 months	C5/6	91
I.B.	40	19 years	C5/6	98
J.P.	35	7 years	C5/6	97
J.M.	22	8 months	C6/7	96

GROUP C: Tetraplegic subjects with atropine

I.J.	21	5 years	C4/5	92
J.M.	22	9 months	C6/7	99
J.C.	19	1 year	C5/6	104
W.M.	28	4 years	C5/6	93
S.H.	27	6 months	C4/5	101
G.S.	19	4 months	C4/5	95

Chapters 7 and 8. Serial blood samples were taken for the measurement of haematocrit by the Coulter counter, and two samples were taken from each subject for the radioimmunoassay of plasma noradrenaline (Henry et al., 1975). Because the availability of this assay was limited, serial estimations of plasma noradrenaline concentrations were not made, but were measured in the basal state, and at 45 minutes after insulin, which should coincide with the peak rise of plasma catecholamines in response to hypoglycaemia (Christensen et al., 1975; Garber et al., 1976). Plasma noradrenaline concentration could not be measured with accuracy below 25 ng/l (pg/ml) with the assay described, but for statistical purposes values less than 25 ng/l were assumed to equal 25 ng/l. The values are expressed as mean $\pm$ 1 S.E.M.

The presence of sweat production was measured in two tetraplegic subjects without atropine (I.J. and P.F.) using the micro-hygrometer method described in Chapter 4.

RESULTS

Clinical manifestations of hypoglycaemia

Subjective symptoms: The tetraplegic subjects experienced only mild neuroglycopenia compared with the normal subjects who experienced typical symptoms of hypoglycaemia within 30 minutes of insulin administration. The group of tetraplegic subjects given atropine (group C) experienced neuroglycopenic symptoms which were more severe and prolonged, and in studies with two of these patients the investigation had to be terminated by giving parenteral glucose because of severe neuroglycopenia.

Haemodynamic changes: In the tetraplegic subjects without atropine (group B) no sudden tachycardia occurred to coincide with the nadir

of the blood glucose (as observed in the normal subjects), but a gradual increase in pulse rate was noted in response to hypoglycaemia in the tetraplegic subjects in group B. The pulse rate rose from a mean basal rate of 62 beats/min (range 50 - 80) to a maximum of 72 beats/min (range 58 - 96), but the time after insulin at which the maximal pulse rate was attained was not consistent within the group. This varied from 25 to 60 minutes after insulin injection, and did not correspond to the nadir of the blood glucose concentration. Basal values of systolic, diastolic and mean blood pressure were subnormal in the supine tetraplegic subjects. A small decrease in both systolic and diastolic pressures was noted following hypoglycaemia, but this was not a consistent response. The typical haemodynamic changes during hypoglycaemia in a tetraplegic subject without atropine are shown in Fig. 6.2 (subject P.F.).

The tetraplegic subjects lack a normal sympathetic cardiac innervation, and in group C the adequacy of cholinergic blockade was confirmed by a sustained elevation in pulse rate following the administration of atropine (Fig. 6.3). The pulse rate rose from a mean basal rate of 61 beats/min (range 36 - 72) to 89 beats/min (range 64 - 100). In three subjects a second dose of atropine was given between 100 and 135 minutes after the initial atropine injection to maintain the increased pulse rate. No alteration in pulse rate was observed in this group which could be attributed to the hypoglycaemia. The blood pressure changes in the tetraplegic subjects given atropine (group C) were variable. Immediately after the induction of cholinergic blockade, there was an initial rise both in systolic and diastolic pressures, followed by a decrease in the systolic and diastolic pressures when insulin was injected. However the temporal sequence

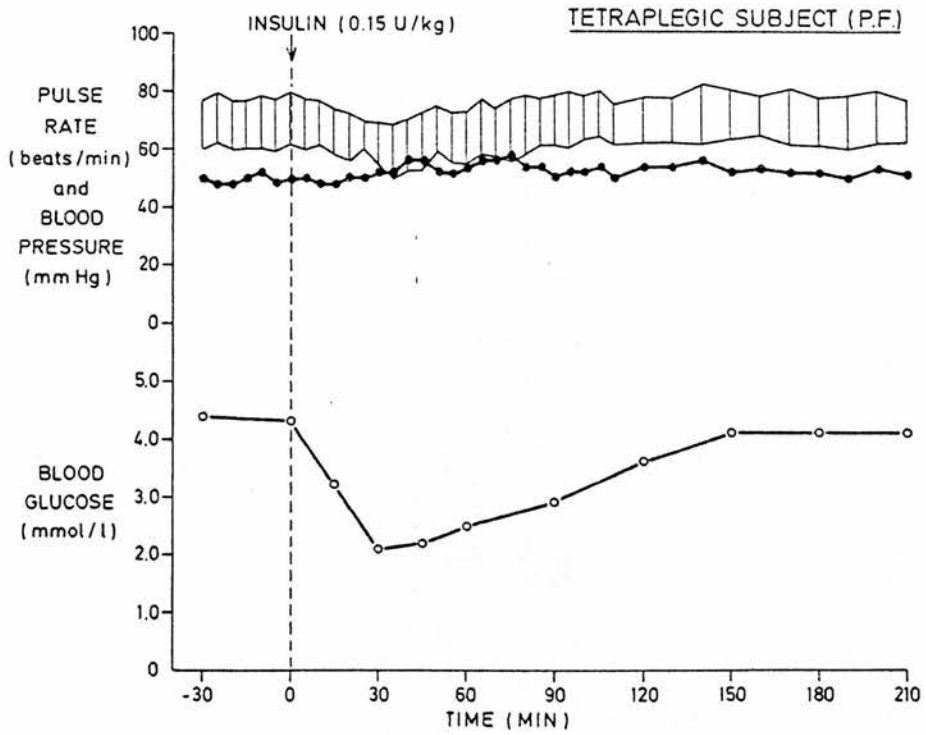

Fig. 6.2 Haemodynamic changes in a tetraplegic subject in response to insulin-induced hypoglycaemia. The changes in pulse rate and blood pressure are shown with the concurrent blood glucose concentrations.

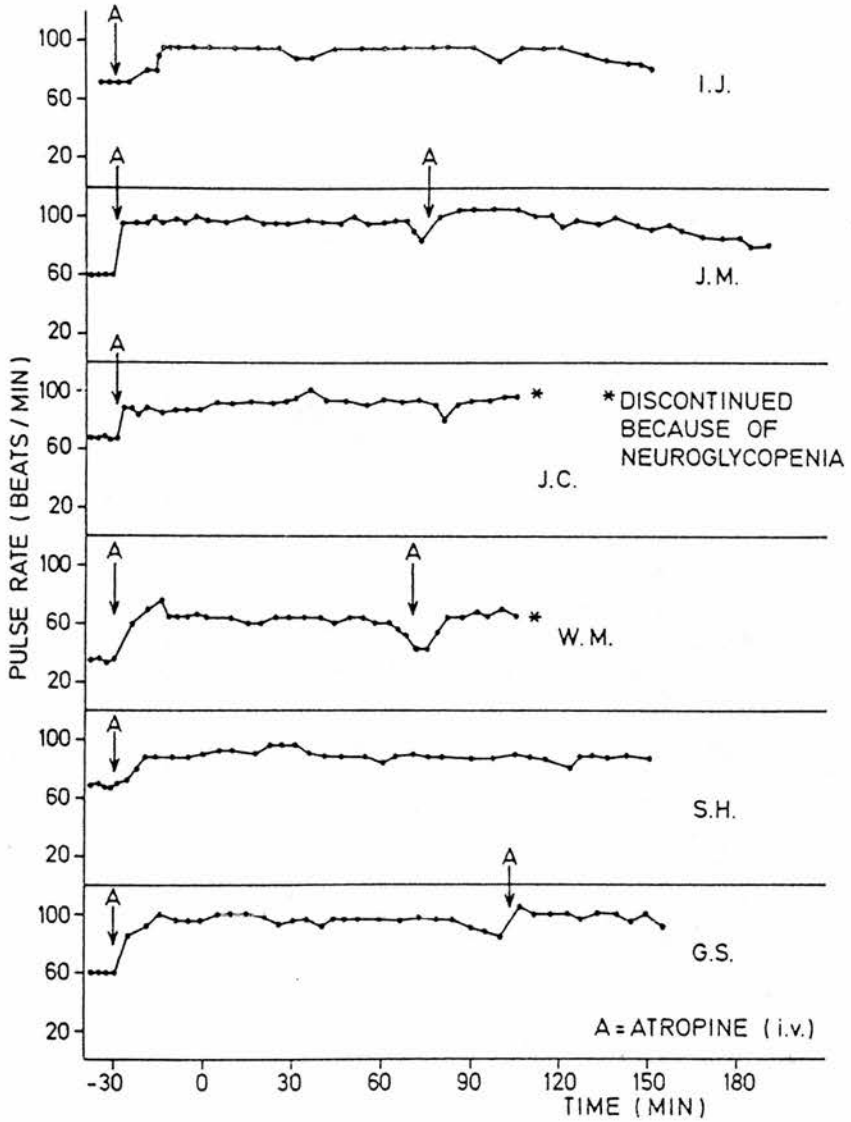

Fig. 6.3 Pulse rate in individual tetraplegic subjects given atropine, which was injected intravenously at points marked A. Insulin was administered at time 0 minutes.

of changes in pulse rate and blood pressure were not consistent within this group.

Haematocrit: The normal rise in haematocrit which is observed in response to hypoglycaemia, was absent in the tetraplegic subjects without atropine (group B) (Fig. 6.4). Haematocrit changes were not measured in group C.

Sweating: Visible sweating was absent in all tetraplegic subjects with or without atropine administration. The measurement of sweat production in two tetraplegic subjects using the micro-hygrometer confirmed the complete absence of sweating in response to hypoglycaemia.

Plasma noradrenaline: Plasma noradrenaline was measured in response to hypoglycaemia in six normal subjects. In this control group, the plasma noradrenaline concentration rose from a mean basal value of 201 $\pm$ 103 ng/l (pg/ml) to 430 $\pm$ 114 ng/l at 45 min after insulin administration (Fig. 6.5). In both tetraplegic groups, the mean basal values were lower than the control group. In the tetraplegic group without atropine (n = 6) the mean basal value was 25 $\pm$ 6 ng/l with a mean value of 31 $\pm$ 6 ng/l at 45 min after insulin. In the tetraplegic group given atropine (n = 6), the mean basal value was 63 $\pm$ 24 ng/l, with a mean value at 45 min of 48 $\pm$ 21 ng/l. There was therefore no demonstrable response to hypoglycaemia in both tetraplegic groups. Using the Wilcoxon rank test, there was a significant difference between the control group and both tetraplegic groups at 45 min after insulin ($p < 0.01$).

DISCUSSION

Evidence for complete disruption of the efferent sympathetic pathway in these tetraplegic subjects is provided by the absence of

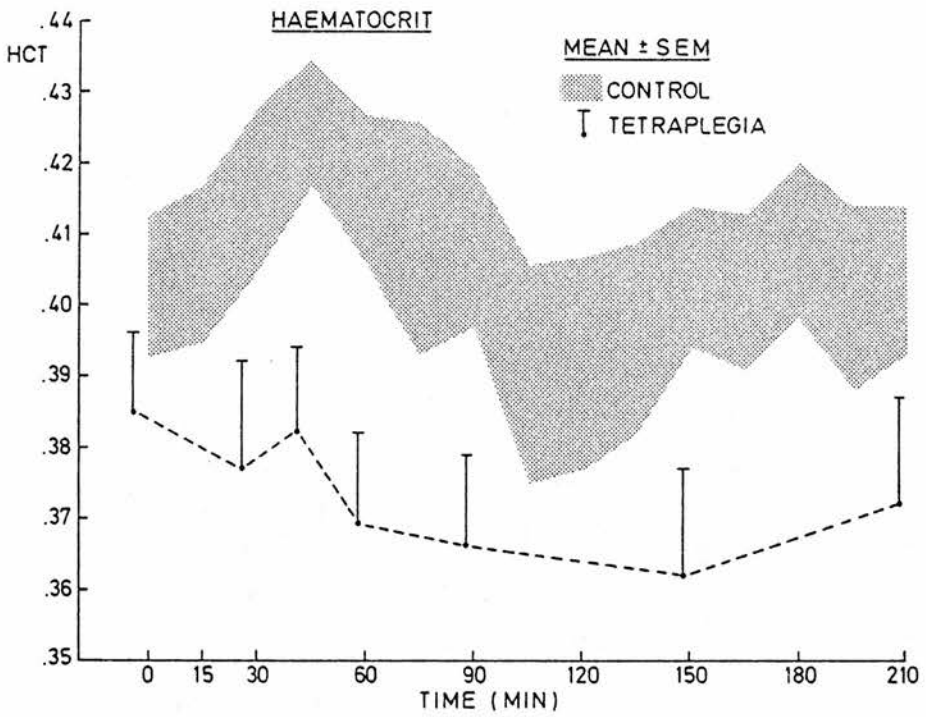

Fig. 6.4 Changes in haematocrit in response to insulin-induced hypoglycaemia in the group of tetraplegic subjects without atropine (group B). The changes in six normal subjects (mean ± SEM) are represented by the shaded area.

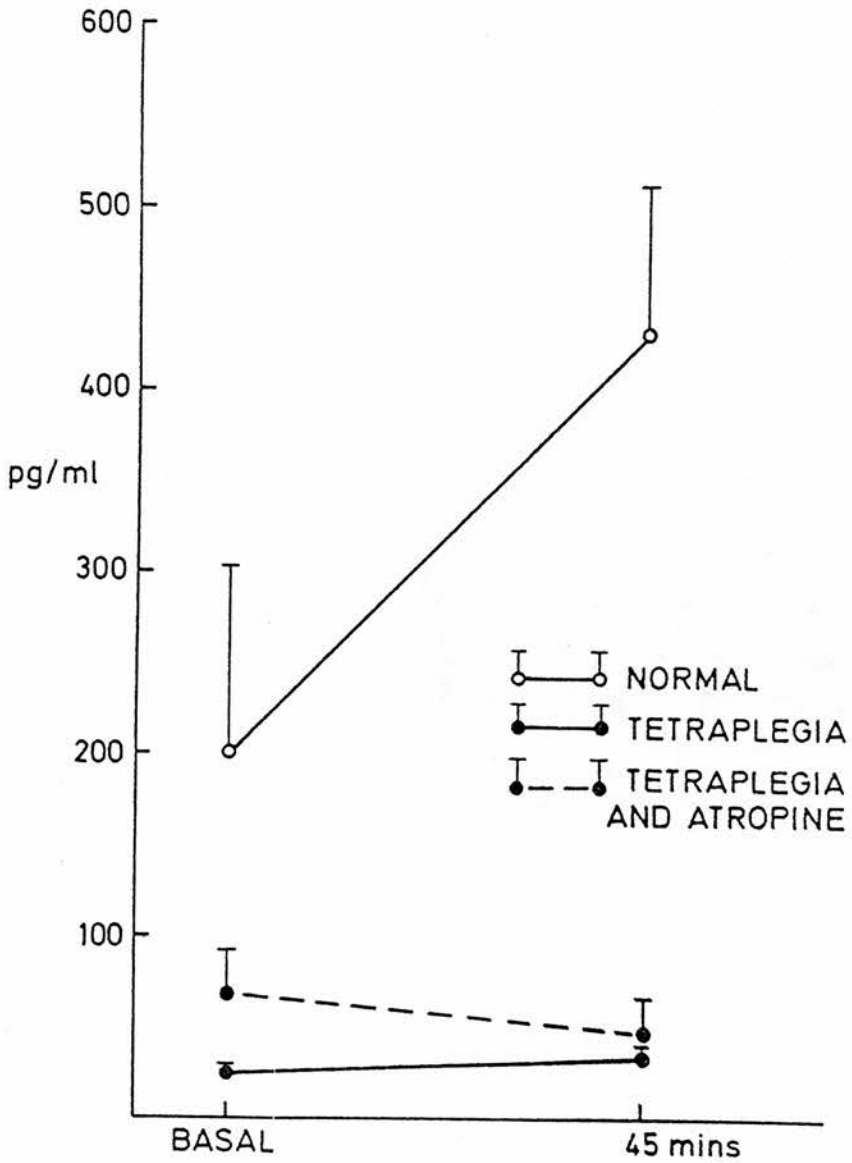

Fig. 6.5 Plasma noradrenaline concentrations (mean ± SEM) in response to hypoglycaemia. Insulin was administered following withdrawal of the basal blood samples. (pg/ml = ng/l).

sweating and tachycardia in response to hypoglycaemia, and by the low basal noradrenaline levels which failed to rise following hypoglycaemia. The latter confirms the findings of Palmer et al., (1976). The normal increase in plasma coagulation factor VIII activity in response to hypoglycaemia, which is mediated via an adrenergic mechanism, is also absent in these tetraplegic subjects (Corrall et al., 1980), which supports the evidence for a total pre-ganglionic sympathectomy. Although the administration of atropine in the described dosage probably does not achieve complete cholinergic blockade, it does allow an assessment of the residual cholinergic mechanisms in these sympathectomised subjects. The adequacy of pharmacological blockade of cardiac cholinergic receptors with atropine was confirmed by the persistent elevation of pulse rate in the tetraplegic subjects.

In normal subjects insulin-induced hypoglycaemia produces a rise in systolic blood pressure, a fall in diastolic pressure and a transient tachycardia (French and Kilpatrick, 1955; Lloyd-Mostyn and Oram, 1975). The normal cardiovascular changes are thought to result from stimulation of the sympatho-adrenal system, associated with an increase in circulating catecholamines and a reduction in neurogenic vasoconstrictor tone (Middleton and French, 1974). These haemodynamic changes are absent following hypoglycaemia in adrenalectomised subjects (Ginsburg and Paton, 1956) and in normal subjects during ganglionic blockade with hexamethonium (Laurence and Stacey, 1952; DiSalvo et al., 1956). However the tachycardia is thought to be unrelated to circulating levels of plasma adrenaline (Christensen et al., 1975), and in patients with splanchnic nerve section with division of the sympathetic nerves below T7, a rise in heart rate was observed in response to hypoglycaemia without a rise in the urinary

excretion of adrenaline (French and Kilpatrick, 1955). The sympathetic cardiac innervation would still be intact in such subjects, and the tachycardia may therefore be the result of increased activity in the cardiac sympathetic nerves. Furthermore, the increase in heart rate is transient in normal subjects and returns to basal levels while the plasma adrenaline concentration is still elevated (Christensen et al., 1975).

Subnormal basal levels of systolic and diastolic blood pressures have been observed previously in tetraplegic subjects (Mathias et al., 1976). In the present study, the decrease both in the systolic and in the diastolic pressures was relatively small in the tetraplegic subjects without atropine, and only a small gradual increase in heart rate was observed. This is consistent however with the rise in heart rate and fall in pulse pressure in tetraplegic patients in response to hypoglycaemia which has been described by Mathias et al., (1979). These investigators surmised that the increase in heart rate was caused by a baroreceptor-induced reflex, mediated by a withdrawal of vagal cardiac tone in response to the fall in blood pressure. The latter may result from an inability to initiate an increase in sympathetic nervous activity by reflex means in response to hypoglycaemia despite a presumed decrease in intravascular volume caused by insulin (Gundersen and Christensen, 1977; Mackay et al., 1978). In the present study there was no significant increase in haematocrit in response to insulin-induced hypoglycaemia in the tetraplegic subjects, so it is possible that a decrease in intravascular volume, secondary to the administration of insulin, does not occur in sympathectomised subjects.

Insulin has been shown to have effects on the cardiovascular system even in the absence of hypoglycaemia. In normal subjects, and

in diabetic subjects without autonomic neuropathy, intravenous insulin can cause an increase in heart rate, particularly in the upright position (Miles and Hayter, 1968; Page et al., 1976). This occurs within five to ten minutes of insulin injection. In diabetic patients with autonomic neuropathy, the administration of insulin can cause profound hypotension (Miles and Hayter, 1968; Page and Watkins, 1976). A decrease in plasma volume and an associated rise of haematocrit in response to insulin was demonstrated in diabetic subjects without autonomic neuropathy, but was absent in diabetic patients with neuropathy (Mackay et al., 1978). It is proposed therefore that the fall in plasma volume promotes the increase in heart rate in these uncomplicated diabetic patients without autonomic neuropathy, since there is a close relationship between the fall of plasma volume and the rise of plasma noradrenaline following insulin (Gundersen and Christensen, 1977). In the present study, both the haematocrit and the plasma noradrenaline concentrations failed to rise in the tetraplegic subjects, so this explanation could not explain the gradual increase in heart rate which was observed. Similarly a normal rise in heart rate after insulin administration has been described in diabetic patients with nephropathy in whom there was no measurable rise in plasma noradrenaline (Christensen et al., 1980). This is consistent with a study which showed that in rabbits the action of insulin occurred independently of the autonomic nervous system (Jacobsen and Christensen, 1979).

The sweating that is a constant feature of the normal hypoglycaemic reaction has been attributed to circulating catecholamines (Freinkel, 1975; Hall et al., 1974). In normal subjects however, the sudden onset of sweating is synchronous with the initial rise in pulse rate (Chapter 4) and precedes the peak in plasma catecholamines

(Christensen et al., 1975). The total absence of sweating in response to hypoglycaemia in the patients with a pre-ganglionic sympathectomy, and the persistence of sweating in adrenalectomised patients (Ginsburg and Paton, 1956) and patients with adrenal denervation (French and Kilpatrick, 1955) suggests that adrenaline is not involved in the sweating response, and favours mediation via a sympathetic cholinergic mechanism.

CHAPTER 7

PLASMA SUBSTRATE RESPONSES TO ACUTE HYPOGLYCAEMIA IN MAN: ADRENERGIC AND CHOLINERGIC MECHANISMS

Chapter 7

Plasma substrate responses to acute hypoglycaemia in man: adrenergic and cholinergic mechanisms

Methods

Results: Blood glucose
Blood lactate
Plasma free fatty acids
Plasma cyclic AMP

Discussion

The metabolic response to acute hypoglycaemia in man has been described in Chapter 1. This is characterised by an initial fall in blood glucose which is restored to basal levels by the activation of hepatic glycogenolysis and gluconeogenesis. These processes are accompanied by a rise in blood lactate levels secondary to muscle glycogenolysis and an elevation of plasma free fatty acids following lipolysis in adipose tissue. Acute hypoglycaemia is associated with activation of the sympatho-adrenal system which produces classical autonomic symptoms, and stimulation of post-ganglionic cholinergic nerves, which has been outlined in Chapter 2.

In this chapter and in Chapter 8, studies are described in which the contributions of adrenergic and cholinergic activity to the homeostatic recovery mechanism from acute hypoglycaemia have been examined in man.

METHODS

The subjects who were described in Chapter 6 were studied using an identical protocol (insulin dose 0.15 units/kg body weight; atropine dose 15 µg/kg).

Group A: Normal subjects (n = 11)

Group B: Tetraplegic subjects (n = 6)

Group C: Tetraplegic subjects given atropine (n = 6)

Blood samples were withdrawn 30 minutes prior to the injection of insulin, and at intervals of 30 minutes for 210 minutes after insulin administration. Serial measurements were made of blood glucose (Hill and Kessler, 1961), blood lactate (Hohorst, 1970), plasma free fatty acids (FFA) (Baird et al., 1967) and plasma cyclic AMP (Brown et al., 1972).

Statistics: Results are expressed as mean ± the standard error of the mean (SEM). Statistical significance between groups of subjects was estimated using Student's t test for unpaired data (between groups) or paired data (within group). The data presented in this chapter was distributed normally about the mean value.

RESULTS

Blood glucose: The changes in mean blood glucose in all three groups of subjects are presented in Table 7.1 and illustrated in Fig. 7.1. Following insulin administration, the rate of fall of blood glucose was slower in the two groups of tetraplegic subjects, both of which were significantly different from the normal group at 30 min ($p < 0.001$), with a lesser degree of hypoglycaemia being attained. At this time (30 min) there was also a significant difference between the two groups of tetraplegic subjects ($p < 0.05$). In the tetraplegic subjects without atropine (group B) blood glucose recovery proceeded at a similar rate to the normal group, and during the recovery phase the small difference between these groups was not statistically significant.

In the tetraplegic subjects given atropine (group C), the blood glucose rise following hypoglycaemia was much slower than in the other two groups, with a significant impairment of blood glucose recovery. There was a significant difference in the mean blood glucose values compared with the normal group at 60 min ($p < 0.01$), 90 min ($p < 0.001$) and 150 min ($p < 0.001$) after insulin. Blood glucose levels in individual tetraplegic subjects given atropine are shown in Fig. 7.2. Blood glucose recovery was impaired in all subjects, and two subjects developed symptoms of severe neuroglycopenia which necessitated discontinuation of the study by the administration of parenteral glucose.

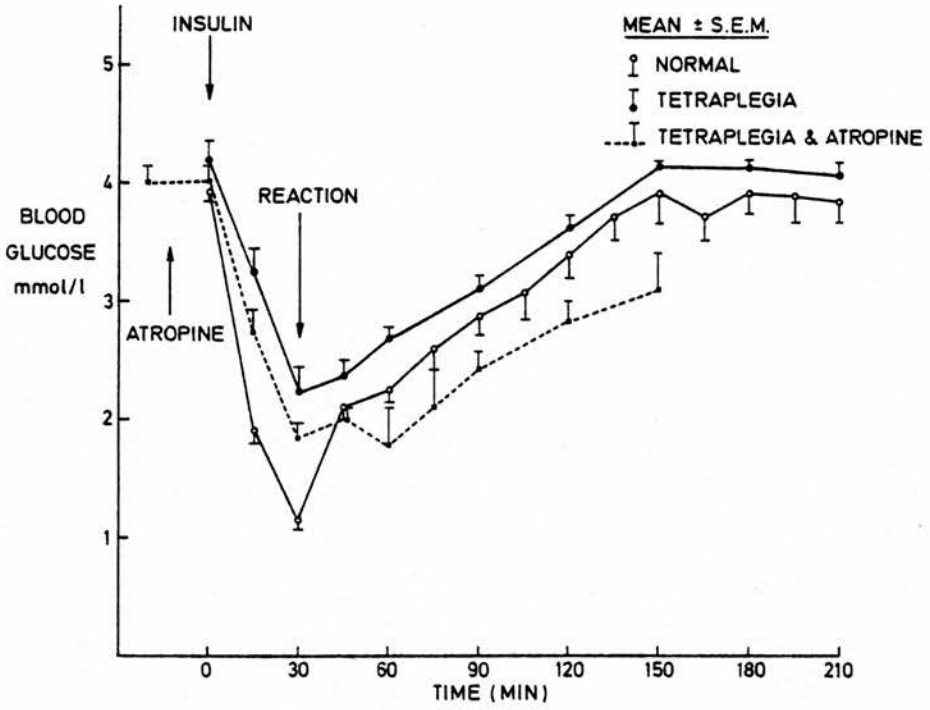

Fig. 7.1 Blood glucose concentrations (mean ± SEM) in normal subjects (n = 11), tetraplegic subjects (n = 6) and tetraplegic subjects given atropine (n = 6) in response to intravenous insulin.

TABLE 7.1

CHANGES OF BLOOD GLUCOSE (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (mmol/l)

INSULIN ADMINISTERED AT 0 MIN

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)	STATISTICAL SIGNIFICANCE p VALUE A vs B	B vs C	A vs C
-30			3.9 ± 0.2			
0	3.9 ± 0.1	4.2 ± 0.2	4.0 ± 0.2			
15	1.9 ± 0.1	3.2 ± 0.2	2.9 ± 0.1	$p < 0.001$	N.S.	$p < 0.001$
30	1.2 ± 0.1	2.2 ± 0.2	1.9 ± 0.1	$p < 0.001$	N.S.	$p < 0.001$
45	2.1 ± 0.1	2.4 ± 0.1	2.0 ± 0.1	N.S.	N.S.	N.S.
60	2.3 ± 0.1	2.7 ± 0.1	1.8 ± 0.3	N.S.	$p < 0.01$	$p < 0.01$
90	2.9 ± 0.2	3.1 ± 0.1	2.4 ± 0.2	N.S.	$p < 0.001$	$p < 0.001$
120	3.4 ± 0.2	3.6 ± 0.1	2.8 ± 0.2	N.S.	$p < 0.001$	$p < 0.001$
150	3.9 ± 0.2	4.2 ± 0.03	3.1 ± 0.3	N.S.	$p < 0.001$	$p < 0.001$
180	3.9 ± 0.2	4.2 ± 0.1		N.S.		
210	3.9 ± 0.2	4.1 ± 0.1		N.S.		

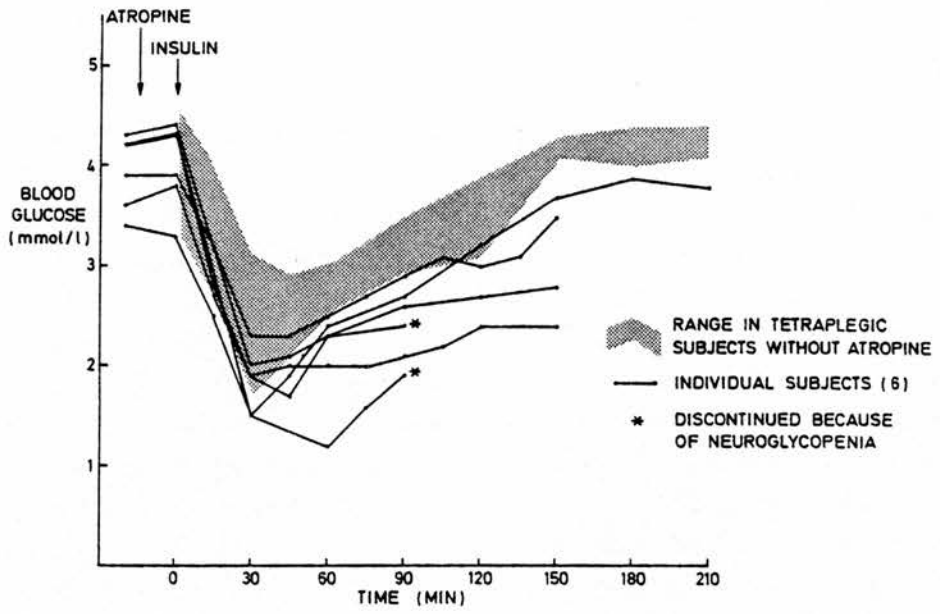

Fig. 7.2 Individual blood glucose concentrations in tetraplegic subjects given atropine (group C) in response to intravenous insulin. The range of blood glucose concentrations in tetraplegic subjects without atropine (group B, n = 6) is shown by the shaded area.

Blood lactate: In the normal subjects blood lactate increased to a peak value at 60 min ($p < 0.001$ vs mean basal value of normal group) then declined to basal levels by 150 min (Fig. 7.3). The mean blood lactate concentrations in the normal group and in the tetraplegic group without atropine (group B) are shown in Table 7.2. In the tetraplegic subjects without atropine, a small rise of blood lactate was observed at 30 min ($p < 0.02$ vs mean basal value), but the mean blood lactate was significantly lower than the normal group at 60 min ($p < 0.05$). Blood lactate concentrations were measured in two tetraplegic subjects given atropine and the individual values are shown in Table 7.2. These values were similar to the levels observed in the tetraplegic group without atropine.

Plasma FFA: The mean values of plasma FFA are shown in Table 7.3 and are depicted in Fig. 7.4. In all three groups the plasma FFA fell after insulin administration for 30 min. A subsequent rise to values above basal was observed in the normal group, and a similar but delayed pattern was present in both tetraplegic groups. This delay was more pronounced in the tetraplegic group given atropine (group C).

Plasma cAMP: Mean plasma cAMP concentrations are shown in Table 7.4 and are illustrated in Fig. 7.5. In the normal group the mean plasma cAMP increased rapidly to a peak more than double the mean basal value at 30 min ($p < 0.01$). This rise was absent in both tetraplegic groups.

The temporal relationship of the changes in these parameters can be compared in all three groups in a composite figure (Fig. 7.6).

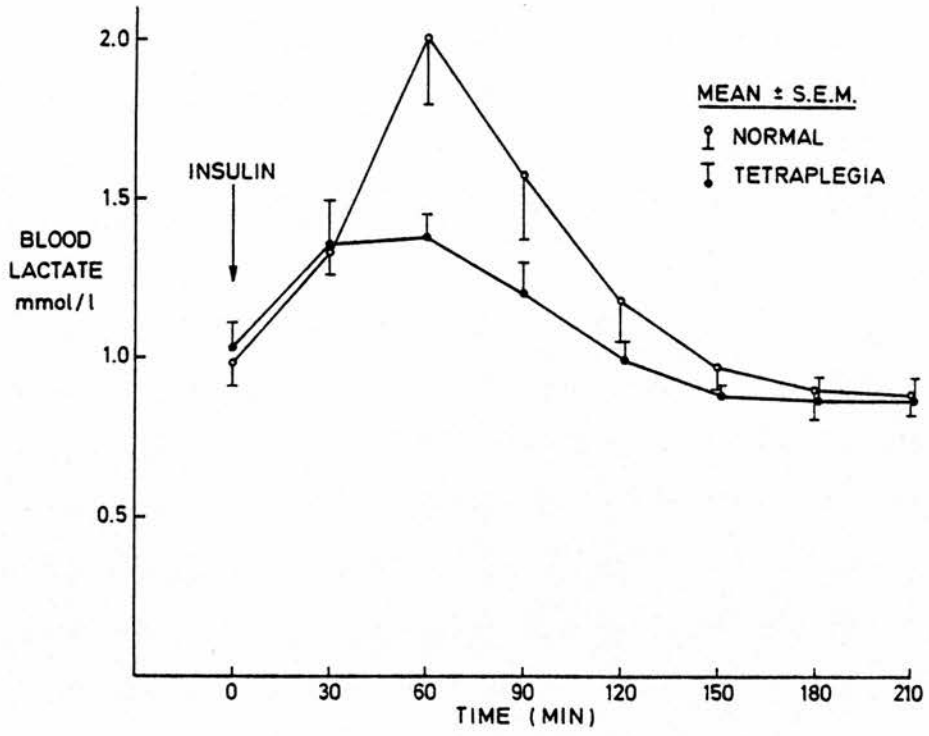

Fig. 7.3 Blood lactate concentrations (mean ± SEM) in normal subjects (n = 11) and tetraplegic subjects (group B, n = 6) in response to insulin-induced hypoglycaemia.

TABLE 7.2

CHANGES OF BLOOD LACTATE (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (mmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	STATISTICAL SIGNIFICANCE p VALUE A vs B	GROUP C (TWO INDIVIDUALS) TETRAPLEGIC SUBJECTS PLUS ATROPINE	
				SUBJECT S.H.	SUBJECT G.S.
0	0.98 ± 0.07	1.03 ± 0.08		0.90	1.08
30	1.33 ± 0.08	1.35 ± 0.14	N.S.	1.50	1.33
60	2.00 ± 0.21	1.38 ± 0.07	$p < 0.05$	1.38	1.50
90	1.57 ± 0.20	1.20 ± 0.10	$p < 0.1$	0.98	1.32
120	1.18 ± 0.13	0.99 ± 0.06	N.S.	0.84	1.18
150	0.97 ± 0.07	0.88 ± 0.03	N.S.		1.06
180	0.90 ± 0.09	0.87 ± 0.07	N.S.		
210	0.89 ± 0.07	0.87 ± 0.07	N.S.		

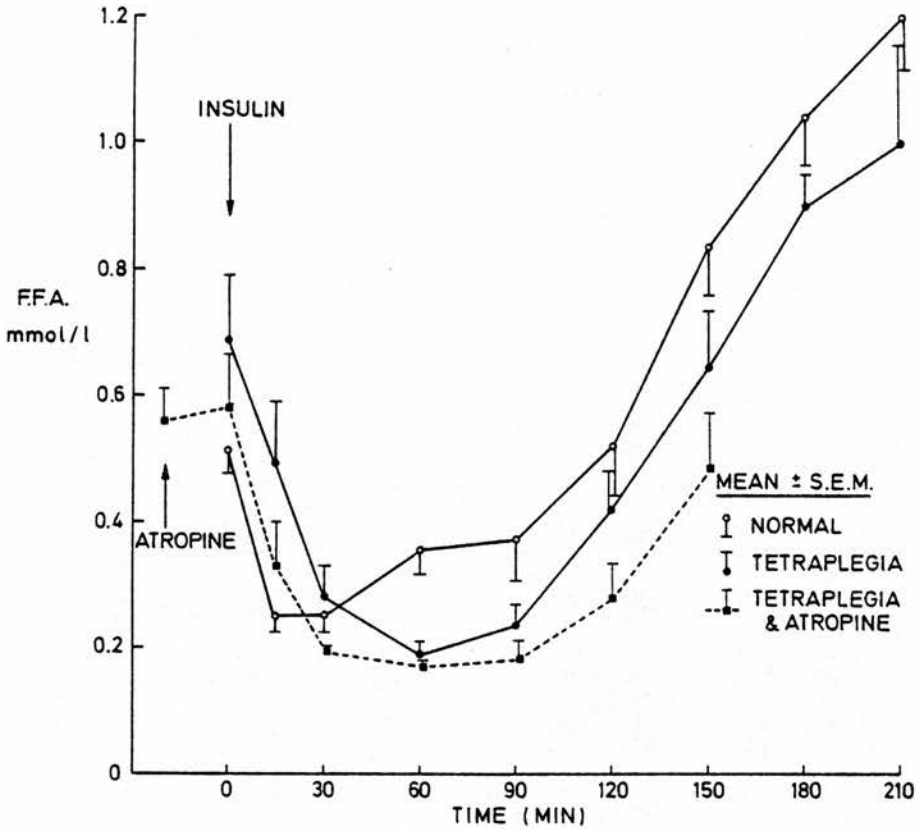

Fig. 7.4 Plasma free fatty acid concentrations (mean ± SEM) in normal subjects (n = 7), tetraplegic subjects (n = 6) and tetraplegic subjects given atropine (n = 5) in response to insulin-induced hypoglycaemia.

TABLE 7.3

CHANGES OF PLASMA FFA (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (mmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 7)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 5)
-30			0.56 ± 0.05
0	0.52 ± 0.03	0.68 ± 0.11	0.58 ± 0.08
15	0.25 ± 0.02	0.49 ± 0.10	0.33 ± 0.07
30	0.25 ± 0.02	0.28 ± 0.05	0.20 ± 0.01
60	0.35 ± 0.04	0.19 ± 0.02	0.17 ± 0.01
90	0.37 ± 0.07	0.23 ± 0.04	0.18 ± 0.03
120	0.52 ± 0.08	0.42 ± 0.06	0.28 ± 0.05
150	0.83 ± 0.08	0.65 ± 0.09	0.48 ± 0.09
180	1.04 ± 0.08	0.90 ± 0.05	
210	1.20 ± 0.09	1.00 ± 0.15	

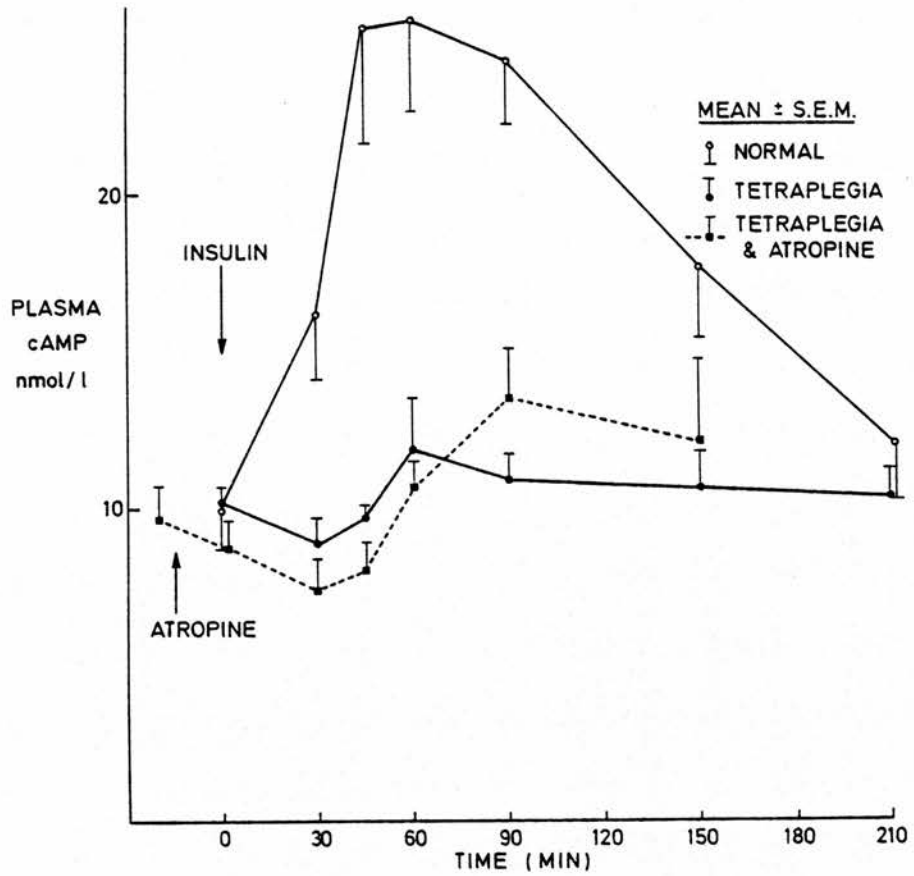

Fig. 7.5 Plasma cyclic AMP concentrations (mean ± SEM) in normal subjects (n = 7), tetraplegic subjects (n = 5) and tetraplegic subjects given atropine (n = 6) in response to insulin-induced hypoglycaemia.

TABLE 7.4

CHANGES OF PLASMA CYCLIC AMP (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (nmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 7)	GROUP B TETRAPLEGIC SUBJECTS (n = 5)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)	STATISTICAL SIGNIFICANCE p VALUE A vs B	A vs C
-30			9.3 ± 1.4		
0	9.9 ± 1.2	10.2 ± 0.5	8.7 ± 0.9		
30	16.2 ± 2.1	8.9 ± 0.8	7.4 ± 1.0	$p < 0.01$	$p < 0.01$
45	25.3 ± 3.7	9.7 ± 0.4	8.0 ± 0.9	$p < 0.01$	$p < 0.01$
60	24.5 ± 2.9	11.9 ± 1.6	10.7 ± 0.8	$p < 0.01$	$p < 0.01$
90	24.2 ± 2.0	10.9 ± 0.8	13.5 ± 1.6	$p < 0.01$	$p < 0.01$
150	17.6 ± 2.2	10.6 ± 1.2	12.1 ± 2.7		
210	11.0 ± 1.8	10.3 ± 0.9			

DISCUSSION

The slower fall in blood glucose following insulin and the lesser degree of hypoglycaemia compared to normal subjects, suggests a relative resistance to insulin in the tetraplegic subjects, and this may be related to chronic inactivity (Lipman et al., 1970). Palmer et al., (1976) attempted to circumvent this relative insensitivity to insulin by administering a larger dose of insulin (0.2 units/kg body weight) to tetraplegic subjects, but were still unable to achieve a degree of hypoglycaemia equivalent to that of normal subjects. In the present study the normal and the tetraplegic subjects received the same dose of insulin (0.15 units/kg body weight). The blood glucose recovery proceeded at a normal rate in the tetraplegic subjects without atropine. The absence of a catecholamine response to hypoglycaemia in these sympathectomised subjects (Chapter 6) does not appear to impede the restoration of normoglycaemia. This is consistent with a normal blood glucose recovery from hypoglycaemia in adrenalectomised subjects (Ginsburg and Paton, 1956; Von Euler et al., 1961; Brodows et al., 1976; Ensinck et al., 1976; Gerich et al., 1979), in sympathectomised subjects (Palmer et al., 1976; Brodows et al., 1976) and in normal subjects during either separate (Clarke et al., 1979) or combined (Rizza et al., 1979a) alpha and beta adrenergic blockade.

Ablative studies in animals have suggested the existence of sub-hypothalamic centres in the cervical and thoracic spinal cord which may initiate a metabolic response to hypoglycaemia (Crone, 1965; Goldfien, 1966). In the present study it is possible that hypoglycaemia could activate a counterregulatory mechanism via a spinal cord reflex below the level of transection. In human subjects with tetraplegia

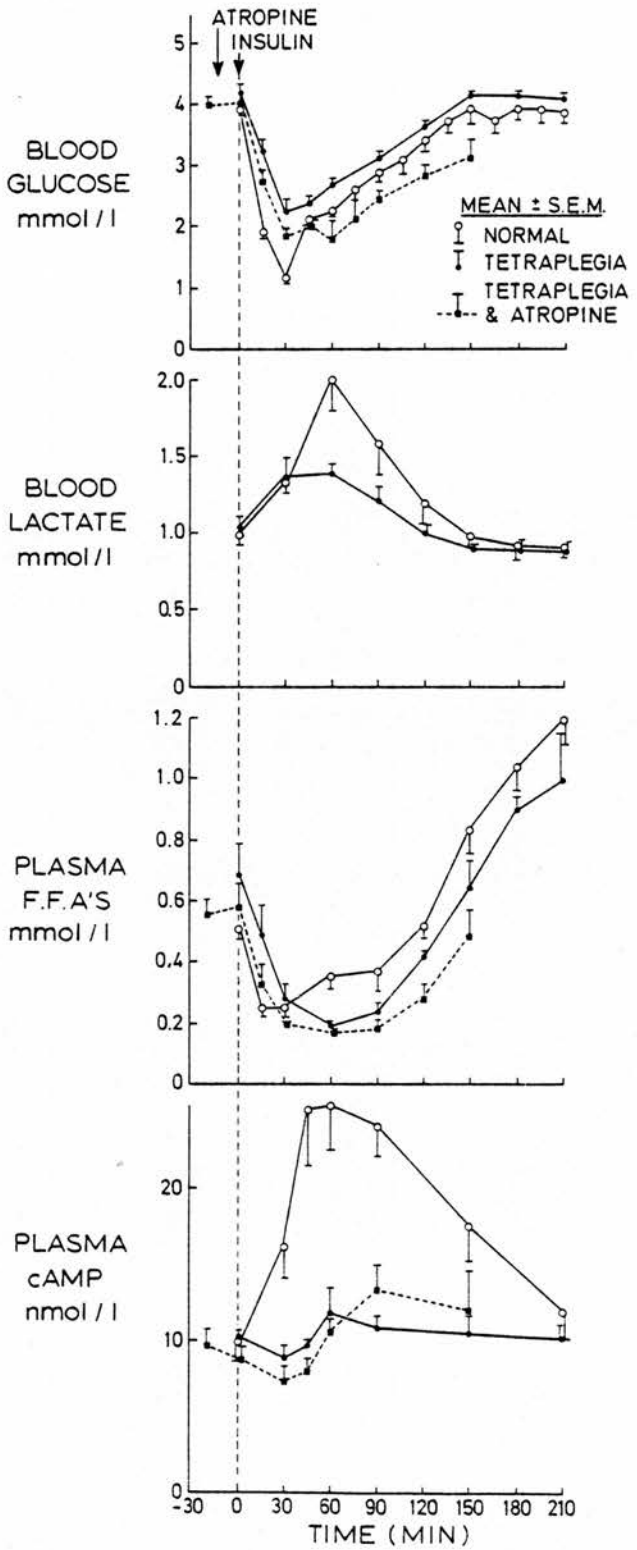

Fig. 7.6 Changes in blood glucose, blood lactate, plasma free fatty acids and plasma cyclic AMP concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects and in tetraplegic subjects with or without atropine. Insulin was administered at time 0 minutes.

however, the infusion of 2-deoxy-D-glucose to induce intracellular glucopenia did not provoke the rise of blood glucose which is observed in normal subjects (Brodows et al., 1973). It is unlikely therefore that a spinal reflex in man could be invoked to explain the normal blood glucose recovery in the tetraplegic subjects without atropine in the present study.

The administration of atropine to the tetraplegic subjects was associated with a greater fall of blood glucose in response to insulin, compared with the tetraplegic group without atropine. Blood glucose recovery also was delayed significantly and this produced severe neuroglycopenia in two of the six tetraplegic subjects.

The rise of blood lactate in normal subjects results from muscle glycogenolysis (Haugaard et al., 1976). Plasma free fatty acids increase when lipolysis is reactivated after an initial inhibition by insulin (Armstrong et al., 1961). The rises of lactate and of free fatty acids following hypoglycaemia are reduced by adrenergic antagonists (Billington et al., 1954; Di Salvo et al., 1956; Werk et al., 1961; Abramson et al., 1966; Abramson and Arky, 1968) and are not observed in response to intracellular glucopenia induced by 2-deoxy-D-glucose in sympathectomised patients (Brodows et al., 1973; Brodows et al., 1975), thus indicating a mediation via catecholamines. In the present study the minimal rise of blood lactate and the delayed rise of plasma free fatty acids in the sympathectomised subjects following hypoglycaemia can be attributed to adrenergic denervation. Although lactate is an important precursor for hepatic gluconeogenesis during the recovery from hypoglycaemia (Exton, 1979; Young and Landsberg, 1977), the impairment of lactate production had no effect on blood glucose recovery in the tetraplegic subjects without atropine, and

cannot therefore be the primary cause of the retarded glucose recovery during cholinergic blockade in such subjects.

The absence of a rise of plasma cyclic AMP in response to hypoglycaemia in all tetraplegic subjects in the present study suggests that the activation of hepatic adenyl cyclase by hypoglycaemia is mediated by catecholamines. The possible role of pancreatic glucagon in this process can be excluded by data presented in Chapter 8, demonstrating no deficiency of glucagon secretion in these tetraplegic subjects in response to hypoglycaemia. In the tetraplegic group without atropine the normal recovery of blood glucose, unaccompanied by a rise in plasma cAMP, is consistent with evidence from in vitro studies which indicate that gluconeogenesis may be activated independently of this nucleotide (Sherline et al., 1972; Tolbert et al., 1973; Kneer et al., 1974; Guder and Rupprecht, 1975), and confirms a previous study in tetraplegic subjects (Brodows et al., 1976).

CHAPTER 8

HORMONAL RESPONSE TO ACUTE HYPOGLYCAEMIA IN MAN: NEURAL CONTROL OF PANCREATIC ISLET CELL FUNCTION AND HORMONAL MEDIATION OF METABOLIC HOMEOSTASIS

Chapter 8

Hormonal response to acute hypoglycaemia in man: neural control of pancreatic islet cell function and hormonal mediation of metabolic homeostasis.

Methods

Results: Plasma insulin
Plasma C-peptide
Plasma pancreatic glucagon
Plasma cortisol
Plasma ACTH
Plasma growth hormone
Plasma pancreatic polypeptide

Discussion

Acute insulin-induced hypoglycaemia in man produces a rise in the circulating levels of several hormones, some of which play an important role in the restoration of normoglycaemia. The effects of the major counterregulatory hormones were reviewed in Chapter 1. The relative importance of each of these hormones to the initiation and control of metabolic recovery from hypoglycaemia has not been defined clearly, nor have the contributions of the sympathetic and parasympathetic neural mechanisms which may influence hormonal secretion.

The hormonal secretion of the pancreatic islets plays a central role in the regulation of carbohydrate homeostasis. Increased alpha cell activity in response to hypoglycaemia results in a pronounced rise in plasma levels of pancreatic glucagon (Gerich et al., 1974), in conjunction with a concomitant decrease in beta cell secretion of insulin which is reflected by a reduction in plasma C-peptide concentrations (Horwitz et al., 1975b; Service et al., 1977; Liljenquist et al., 1978). Pancreatic islet cell function may also be influenced by circulating catecholamines and by adrenergic and cholinergic neural activity.

The role of the autonomic innervation in the regulation of the metabolic response to hypoglycaemia is examined further in this chapter, with respect to the effect of adrenergic and cholinergic mechanisms on pancreatic islet cell function and on the secretion of counterregulatory hormones.

METHODS

The same three groups of subjects (Group A - normal; Group B - tetraplegic; Group C - tetraplegic plus atropine) were used as described

in Chapter 6 (Table 6.1) using an identical protocol. The same dose of insulin (0.15 units/kg body weight) was administered to all subjects and basal blood samples were withdrawn prior to the induction of cholinergic blockade in Group C, and 30 minutes before the injection of insulin in Groups A and B. Serial blood sampling was performed at 30 minute intervals throughout the study to 210 minutes after insulin administration.

Serial measurements were made of plasma insulin (Ashby and Speake, 1975), plasma C-peptide (Heding, 1975), plasma pancreatic glucagon (C-terminal glucagon-like immunoreactivity) (Stout et al., 1976), plasma cortisol (Mattingly, 1962), plasma growth hormone (GH) (Hunter, 1976) and plasma pancreatic polypeptide (PP) (Adrian et al., 1976). Plasma ACTH was measured using a double antibody radioimmunoassay on unextracted plasma (Ratcliffe, J.G. and Gray, C.E., unpublished).* (In this study, plasma pancreatic glucagon was assayed by Professor K.D. Buchanan's laboratory).

Statistics: Results are expressed as mean ± the standard error of the mean (SEM). Statistical significance between groups of subjects was estimated using Student's t test for paired (within group) or unpaired (between group) data, except when data was not normally distributed about the mean value. The Wilcoxon rank test was used to assess statistical significance for pancreatic glucagon, GH and ACTH which were not distributed in a normal manner.

*See appendix for ACTH assay.

RESULTS

Plasma insulin: Following the administration of exogenous insulin, plasma insulin rose to similar peak levels in all three groups of subjects, followed by a similar exponential decline (Fig. 8.1). There was no significant difference between the three groups at each time point, and the mean values for all groups are shown in Table 8.1.

Plasma C-peptide: The mean plasma C-peptide levels for all groups are shown in Fig. 8.2 and Table 8.2. The mean basal C-peptide levels were significantly higher in both groups of tetraplegic subjects compared to the normal group (group B vs A, $p < 0.001$; group C vs A, $p < 0.05$), but the mean basal values of groups B and C were not significantly different. Mean plasma C-peptide fell following insulin administration in all three groups. In the normal subjects the mean plasma C-peptide concentration decreased to the effective detection limit of the assay (0.06 nmol/l) by 60 min after insulin, and had not returned to the normal basal level by 210 min, despite the restoration of normoglycaemia. A similar pattern was observed in the tetraplegic subjects without atropine, but the mean plasma C-peptide levels were significantly higher than in the normal group ($p < 0.001$) from 60 min onwards, consistent with the lesser degree of hypoglycaemia attained in this group. An intermediate response was observed in the tetraplegic group given atropine, with a decline in the mean plasma C-peptide level to the effective detection limit at 150 minutes.

Plasma pancreatic glucagon: The changes of mean plasma pancreatic glucagon levels are shown in Table 8.3, and are depicted in Fig. 8.3. Plasma pancreatic glucagon rose in response to hypoglycaemia in

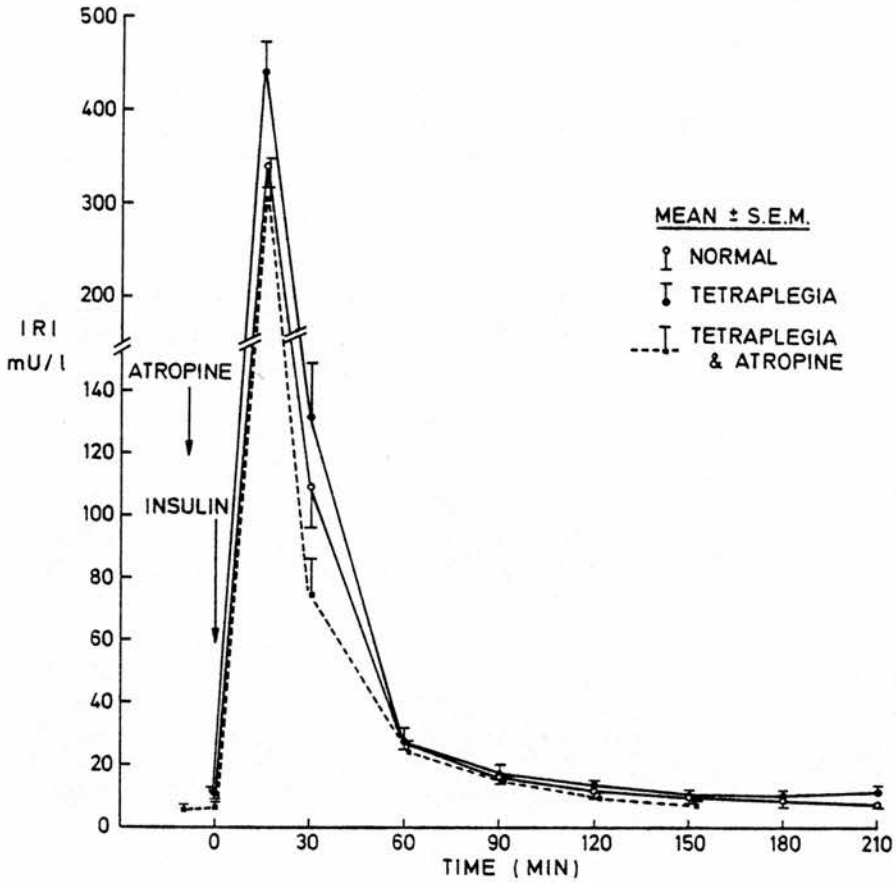

Fig. 8.1 Plasma insulin concentrations (mean ± SEM) following intravenous injection of exogenous short-acting insulin in normal subjects (n = 11), tetraplegic subjects (n = 6) and tetraplegic subjects given atropine (n = 6). (IRI = immunoreactive insulin).

TABLE 8.1

PLASMA INSULIN (MEAN ± SEM) FOLLOWING INJECTION OF EXOGENOUS INSULIN AT TIME 0 MIN (mU/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
-30			4.9 ± 1.8
0	10.4 ± 1.2	11.1 ± 2.0	6.0 ± 1.4
15	341 ± 23	442 ± 30	336 ± 36
30	109 ± 13	131 ± 18	84 ± 9
60	27 ± 2	27 ± 5	26.2 ± 1.1
90	15.6 ± 1.8	18.2 ± 1.8	15.8 ± 1.4
120	12.1 ± 2.4	13.7 ± 0.9	9.2 ± 1.5
150	10.1 ± 2.1	10.5 ± 0.9	7.0 ± 1.4
180	9.0 ± 1.6	10.8 ± 1.1	
210	8.3 ± 1.3	11.7 ± 1.6	

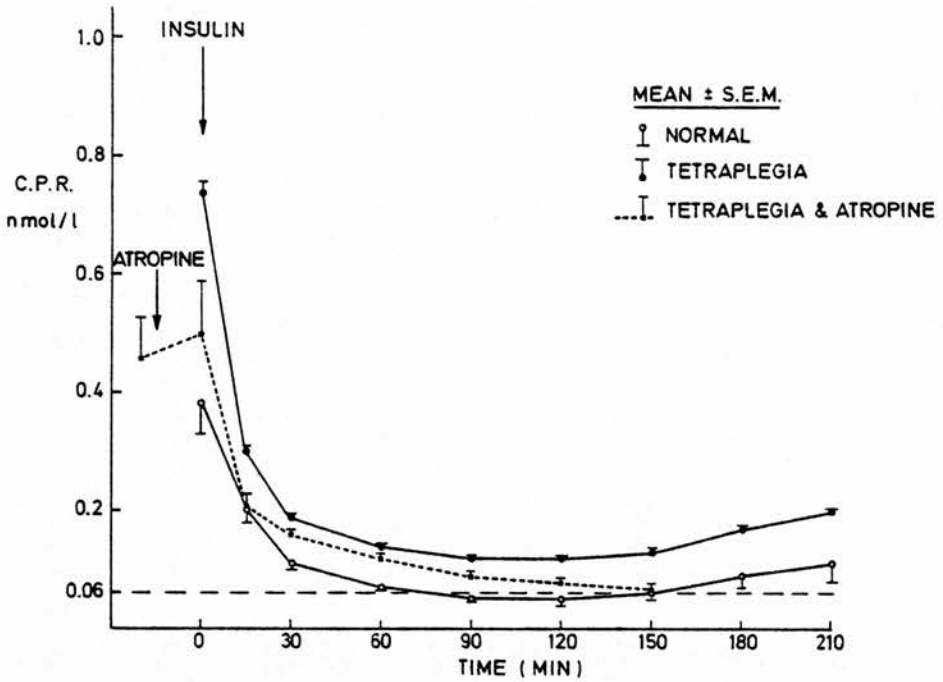

Fig. 8.2 Plasma C-peptide concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 11), tetraplegic subjects (n = 6) and tetraplegic subjects given atropine (n = 6). (C.P.R. = C-peptide immunoreactivity). The effective detection limit of the assay is shown (dashed line).

TABLE 8.2

PLASMA C-PEPTIDE (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (nmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
-30			0.46 ± 0.07
0	0.39 ± 0.05	0.74 ± 0.17	0.50 ± 0.09
15	0.20 ± 0.02	0.30 ± 0.06	0.21 ± 0.02
30	0.11 ± 0.01	0.19 ± 0.03	0.16 ± 0.01
60	0.07 ± 0.01	0.14 ± 0.03	0.12 ± 0.01
90	0.05 ± 0.01	0.12 ± 0.03	0.09 ± 0.01
120	0.05 ± 0.01	0.11 ± 0.03	0.08 ± 0.01
150	0.06 ± 0.01	0.13 ± 0.03	0.07 ± 0.01
180	0.09 ± 0.02	0.17 ± 0.03	
210	0.11 ± 0.03	0.20 ± 0.02	

groups A and B with peak values at 60 minutes. In group C (tetraplegia with atropine), the pancreatic glucagon secretory response was prolonged, reaching a peak value at 90 min, which was not significantly greater than the normal group ($p < 0.1$) but was greater than the tetraplegic group without atropine ($p < 0.02$). The percentage increments of mean pancreatic glucagon from basal to 60 min in the three groups of subjects (group A = 122%; group B = 41%; group C = 63%) were proportional to the percentage fall in mean blood glucose from the basal value to its nadir in the same groups (group A = 71%; group B = 47%; group C = 54%). Blood glucose data has been presented in Chapter 7 (Table 7.1).

Plasma cortisol: A rise of plasma cortisol following hypoglycaemia was observed in all three groups of subjects (Table 8.4, Fig. 8.4). This rise was very similar in the normal subjects and the tetraplegic subjects without atropine. In the tetraplegic subjects given atropine (group C), despite prolonged hypoglycaemia, mean plasma cortisol was lower at 60 min, although this difference failed to achieve statistical significance with either group A or B ($p < 0.1 > 0.05$). The difference was significant at 90 min compared to the normal group ($p < 0.01$) but failed to achieve significance compared to the tetraplegic group without atropine.

Plasma ACTH: In the normal group and in the tetraplegic group without atropine, plasma ACTH levels rose to a peak value at 45 min (Fig. 8.5, Table 8.5). A different pattern of secretion was observed in the tetraplegic group given atropine (group C), with a slower rise to a peak at 60 min, and a higher mean value than both other groups at 90 min, but this did not achieve statistical significance.

Plasma GH: In the normal group the expected rise in plasma GH was observed following hypoglycaemia (Fig. 8.6). In both tetraplegic groups,

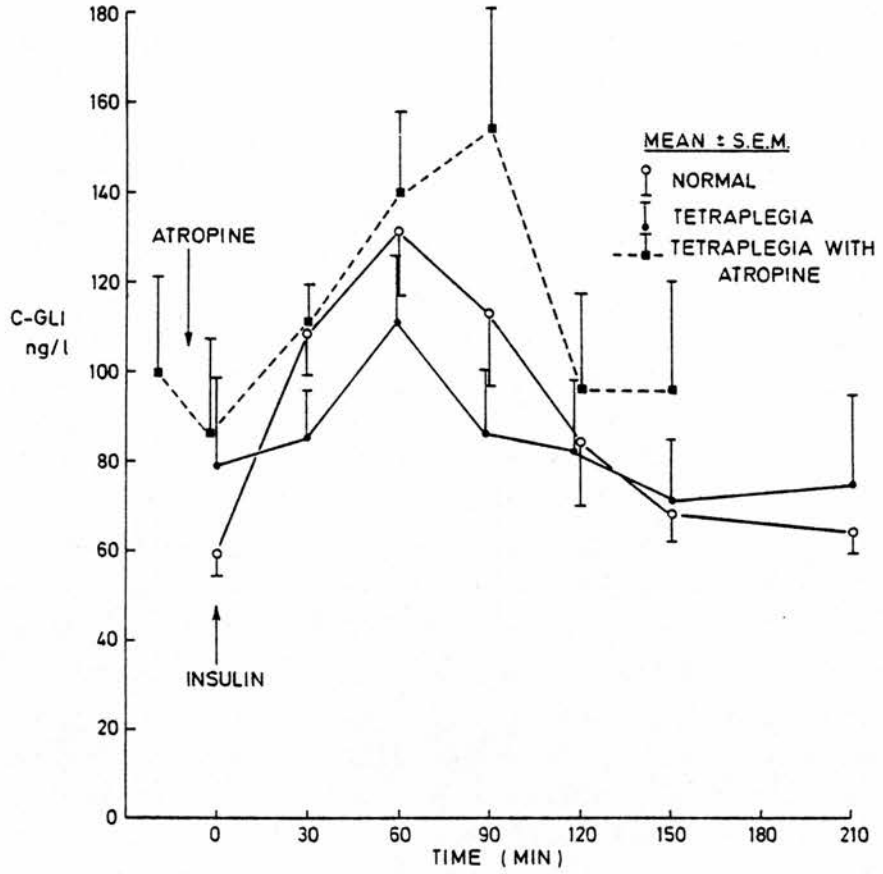

Fig. 8.3 Plasma pancreatic glucagon (C-GLI) concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 10), tetraplegic subjects (n = 6) and tetraplegic subjects given atropine (n = 6).

TABLE 8.3

PLASMA PANCREATIC GLUCAGON (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (ng/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 10)	GROUP B TETRAPLEGIC SUBJECTS (n = 6)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
-30			100 ± 23
0	59 ± 5	79 ± 19	86 ± 24
30	108 ± 9	85 ± 10	111 ± 12
60	131 ± 15	111 ± 15	140 ± 18
90	113 ± 17	86 ± 14	154 ± 32
120	84 ± 14	82 ± 17	96 ± 23
150	68 ± 6	71 ± 14	96 ± 27
210	64 ± 5	76 ± 21	

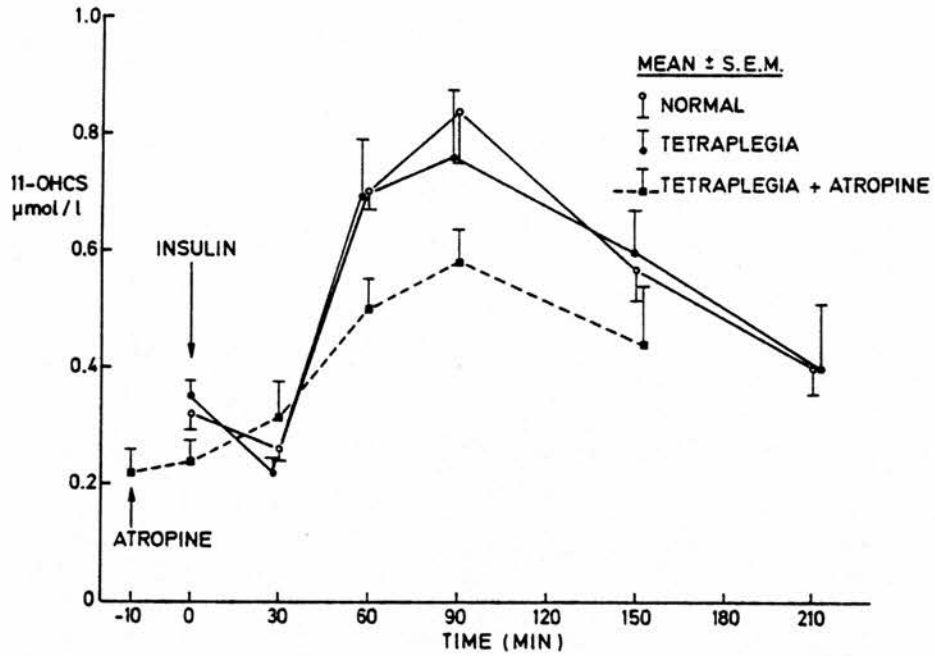

Fig. 8.4 Plasma cortisol concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 11), tetraplegic subjects (n = 5) and tetraplegic subjects given atropine (n = 6). (11-OHCS = 11-hydroxy-corticosteroids).

TABLE 8.4

PLASMA CORTISOL (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (μmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 5)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
-30			0.22 ± 0.04
0	0.32 ± 0.03	0.35 ± 0.03	0.24 ± 0.04
30	0.26 ± 0.02	0.22 ± 0.03	0.32 ± 0.06
60	0.70 ± 0.03	0.69 ± 0.10	0.50 ± 0.05
90	0.84 ± 0.09	0.76 ± 0.13	0.58 ± 0.06
150	0.57 ± 0.06	0.60 ± 0.07	0.44 ± 0.09
210	0.40 ± 0.05	0.40 ± 0.11	

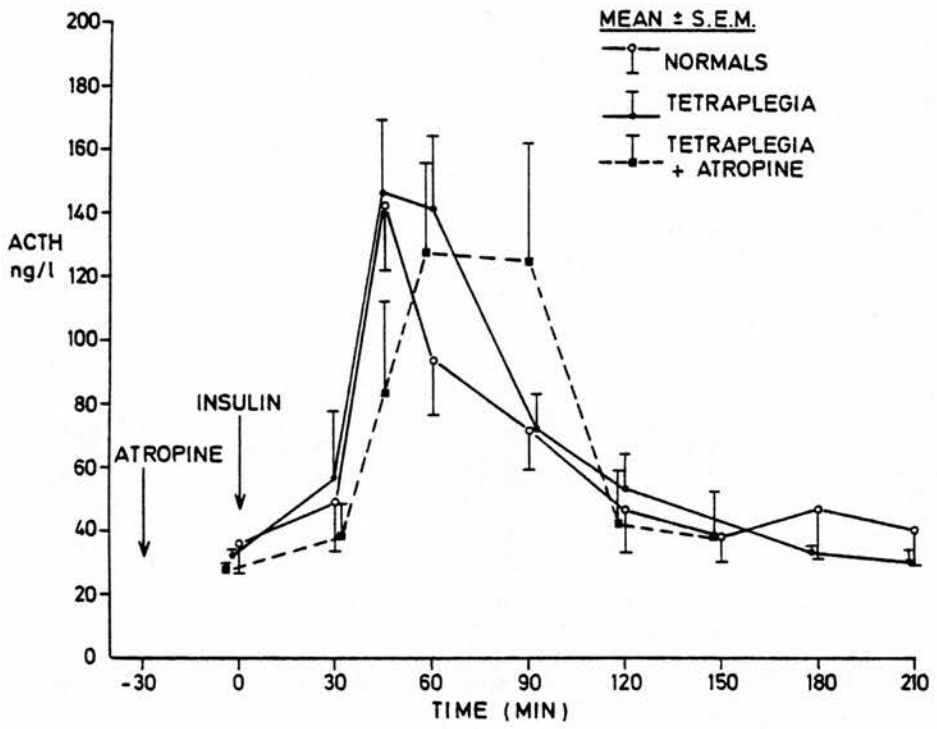

Fig. 8.5 Plasma ACTH concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 9), tetraplegic subjects (n = 4) and tetraplegic subjects given atropine (n = 6).

TABLE 8.5

PLASMA ACTH (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (ng/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 9)	GROUP B TETRAPLEGIC SUBJECTS (n = 4)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
0	36 ± 9	32 ± 1	28 ± 2
30	49 ± 15	57 ± 21	39 ± 10
45	142 ± 20	146 ± 23	84 ± 28
60	93 ± 17	141 ± 23	128 ± 28
90	71 ± 12	71 ± 12	125 ± 37
120	46 ± 13	53 ± 11	42 ± 17
150	38 ± 8		38 ± 14
180	46 ± 15	33 ± 1	
210	40 ± 11	30 ± 4	

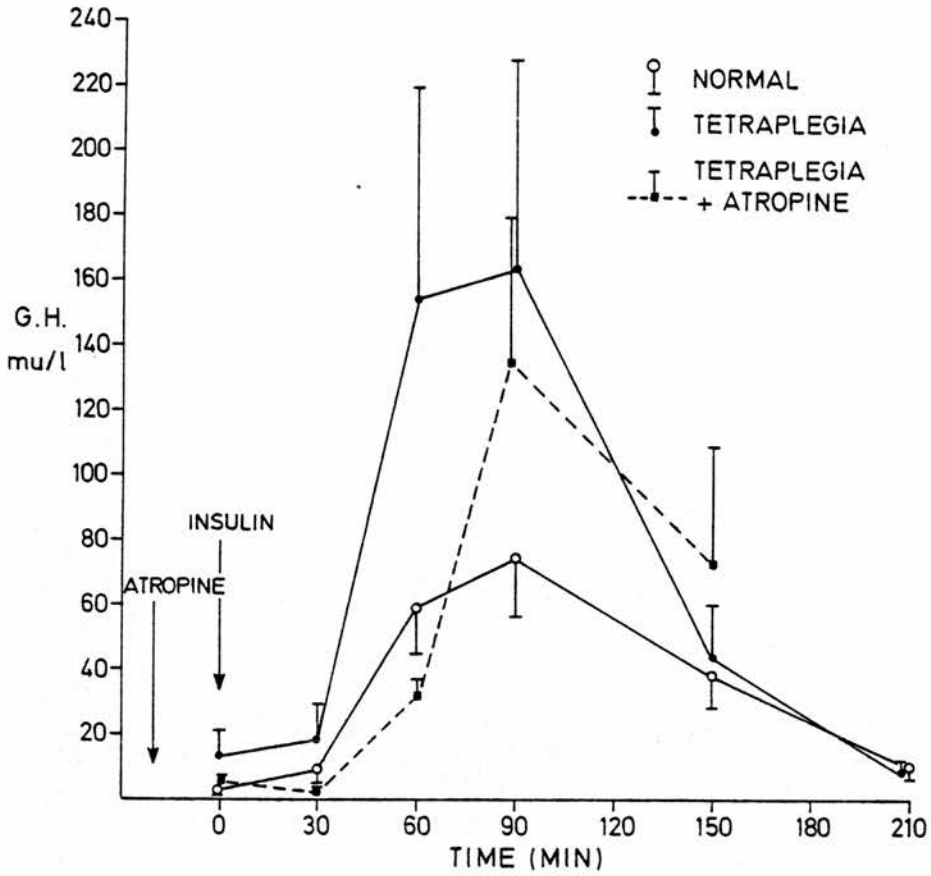

Fig. 8.6 Plasma growth hormone (GH) concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 11), tetraplegic subjects (n = 5) and tetraplegic subjects given atropine (n = 6).

TABLE 8.6

PLASMA GROWTH HORMONE (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (mU/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 11)	GROUP B TETRAPLEGIC SUBJECTS (n = 5)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
0	2 ± 0.6	13 ± 8	5 ± 2
30	9 ± 4	19 ± 10	2 ± 0.6
60	59 ± 14	154 ± 65	31 ± 6
90	74 ± 18	163 ± 65	135 ± 44
150	38 ± 10	44 ± 16	72 ± 37
210	10 ± 3	9 ± 3	

the peak mean values of GH at 60 min were more than double that of the normal group although this was not statistically significant. The mean values for all three groups are documented in Table 8.6.

The concurrent changes in plasma glucagon, cortisol, ACTH and GH are shown in a composite figure (Fig. 8.7).

Plasma PP: In the normal subjects (group A) a marked rise of mean plasma PP concentrations was observed in response to hypoglycaemia, reaching a peak 60 min after insulin administration (Fig. 8.8 Table 8.7). A similar rise was observed in the group of tetraplegic subjects without atropine (group B) although the mean peak value attained was lower, which is compatible with the lesser degree of hypoglycaemia achieved in this group (Fig. 7.1). The plasma PP did not rise in the group of tetraplegic subjects given atropine (group C). One tetraplegic subject who had undergone a previous vagotomy for treatment of a peptic ulcer was also studied (without atropine). There was no measurable rise of PP in response to hypoglycaemia in this subject. In view of his known vagotomy this tetraplegic subject was not included in group B.

DISCUSSION

In the present study the adrenergic and cholinergic mechanisms which may influence human pancreatic islet cell activity, and the counterregulatory hormonal response to hypoglycaemia, have been examined *in vivo*. It is possible that the administration of atropine in this dosage (15 µg/kg body weight) may not achieve complete cholinergic blockade of the pancreatic islets. However, the sustained tachycardia in the absence of sympathetic cardiac innervation suggests that a significant degree of cholinergic blockade with atropine was achieved. The release of pancreatic polypeptide in response to hypoglycaemia has been shown

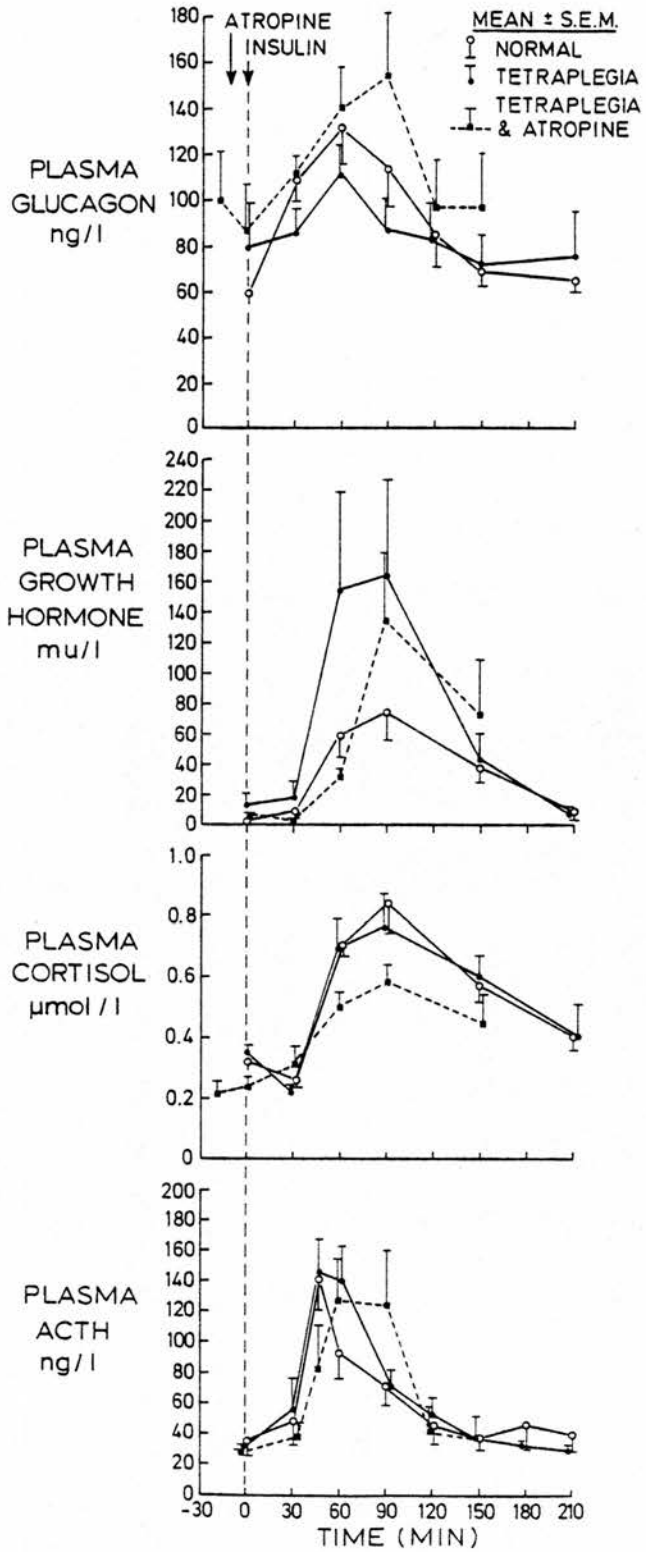

Fig. 8.7 Changes in plasma pancreatic glucagon, growth hormone, cortisol and ACTH (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects and in tetraplegic subjects with or without atropine. Insulin was injected at time 0 minutes.

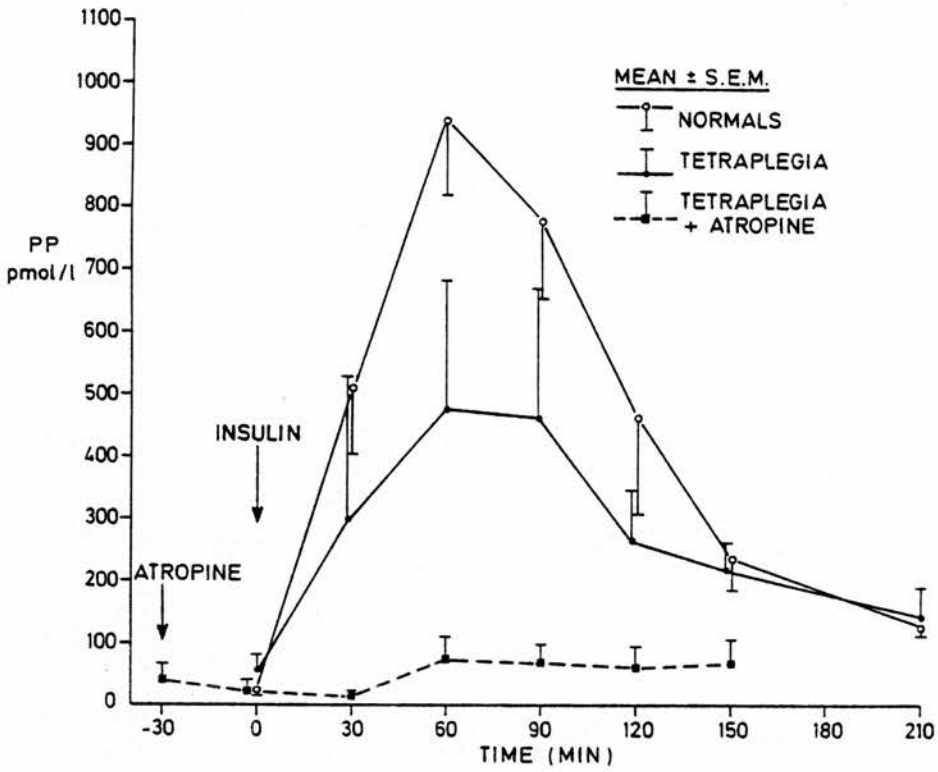

Fig. 8.8 Plasma pancreatic polypeptide (PP) concentrations (mean ± SEM) in response to insulin-induced hypoglycaemia in normal subjects (n = 6), tetraplegic subjects (n = 5) and tetraplegic subjects given atropine (n = 6).

TABLE 8.7

PLASMA PANCREATIC POLYPEPTIDE (MEAN ± SEM) IN RESPONSE TO INSULIN-INDUCED HYPOGLYCAEMIA (pmol/l)

TIME (min)	GROUP A NORMAL SUBJECTS (n = 6)	GROUP B TETRAPLEGIC SUBJECTS (n = 5)	GROUP C TETRAPLEGIC SUBJECTS PLUS ATROPINE (n = 6)
-30			44 ± 19
0	24 ± 6	54 ± 28	24 ± 11
30	508 ± 103	297 ± 235	12 ± 4
60	935 ± 120	472 ± 210	74 ± 33
90	776 ± 129	467 ± 164	71 ± 27
120	465 ± 156	268 ± 77	61 ± 35
150	235 ± 48	218 ± 43	69 ± 41
210	124 ± 11	139 ± 46	

previously to be mediated via vagal cholinergic stimulation (Schwartz et al., 1976; Adrian et al., 1977; Floyd et al., 1977; Schwartz et al., 1978). The abolition of the rise of plasma PP in the tetraplegic subjects given atropine in the present study, confirms the role of a vagal cholinergic mechanism in the control of PP secretion, and supports the production of a significant degree of cholinergic blockade with this dose of atropine.

Despite the limitations of his microscopy, Langerhans (1869) observed that the pancreatic islets were supplied by nerve fibres, and modern fluorescent and histochemical techniques have demonstrated the presence of adrenergic and cholinergic nerve fibres supplying the islet cells (Bencosme, 1959; Woods and Porte, 1974). Studies in animals have demonstrated that stimulation of the parasympathetic nervous system promotes insulin secretion, whereas pancreatic beta cell activity is inhibited both by sympathetic nerve stimulation and by catecholamines acting via an alpha-adrenergic receptor mechanism (Woods and Porte, 1974). In the present study the decline of plasma C-peptide concentration following hypoglycaemia was observed in both groups of tetraplegic subjects, and was commensurate with the changes of blood glucose. It is apparent therefore that pancreatic beta cell suppression in response to hypoglycaemia in man may occur even in the absence of normal sympathetic innervation and in the presence of concomitant pharmacological blockade. The impairment of blood glucose recovery in the tetraplegic subjects given atropine, cannot be explained by a failure of the prolonged inhibition of insulin secretion which normally accompanies the restoration of normoglycaemia (Horwitz et al., 1975b; Liljenquist et al., 1978).

Several studies have shown that pancreatic glucagon secretion is stimulated <u>in vitro</u> by adrenergic agonists (Iversen, 1973;

Pagliara et al., 1974; Weir et al., 1974), and in vivo by the infusion of catecholamines (Gerich et al., 1973) or by neural stimulation (Frohman and Bernardis, 1971; Marliss et al., 1973a) Kaneto et al., 1975). The work of Bloom et al., (1974a) in the calf suggested a cholinergic control of pancreatic glucagon secretion via the vagus nerve, and in this species the secretion of glucagon in response to hypoglycaemia was delayed significantly by atropine. The pancreatic glucagon response to acute hypoglycaemia was reduced moderately in human subjects who had undergone truncal vagotomies, and this was interpreted to indicate cholinergic control of pancreatic alpha cell activity in man (Bloom et al., 1974b). This finding was not confirmed by Palmer et al., (1979) who showed that neither vagotomy nor cholinergic blockade had an effect on the secretion of pancreatic glucagon in man in response to hypoglycaemia.

The previous findings of normal pancreatic glucagon release during hypoglycaemia in patients with a pre-ganglionicsympathectomy (Palmer et al., 1976), and in normal subjects under combined alpha- and beta-adrenergic blockade (Rizza et al., 1979a), are confirmed by this study. This suggests that the sympathetic nervous system plays little part in modulating the secretion of pancreatic glucagon in response to hypoglycaemia, and supports previous studies which suggested that adrenergic mechanisms are not the prime mediators of pancreatic glucagon release following hypoglycaemia in man (Walter et al., 1974; Ensinck et al., 1976). In the present study the rise of plasma pancreatic glucagon concentration was proportional to the degree of hypoglycaemia attained in each group of tetraplegic subjects, and was augmented appropriately during the prolonged hypoglycaemia which occurred with cholinergic blockade. This indicates that under specific conditions, appropriate pancreatic islet cell responses may be observed

despite adrenergic denervation and pharmacological blockade of cholinergic neural mechanisms of control. Isolated pancreatic alpha cells in tissue culture can be stimulated to release glucagon by low concentrations of glucose (Marliss et al., 1973b), suggesting that hypoglycaemia may exert a direct effect via glucopenia.

Peptidergic neurones are contained within the vagus nerve and within the extensive plexus of nerve fibres in the pancreas (Polak and Bloom, 1978). Somatostatin-like immunoreactivity has been demonstrated in peripheral adrenergic neurones in the guinea-pig (Hokfelt et al., 1977) and in the D cells of human pancreatic islets (Polak et al., 1975). Furthermore, plasma somatostatin levels are raised during the recovery from insulin-induced hypoglycaemia in man (Wass et al., 1980). Vasoactive intestinal polypeptide (VIP) has been found in fibres of the vagus nerve (Polak and Bloom, 1978; Fahrenkrug et al., 1978), and is released in response to various physiological stimuli, including direct electrical stimulation of the vagus nerve. VIP has also been shown to stimulate pancreatic glucagon secretion in the perfused cat pancreas (Said, 1978). It is possible therefore that peptidergic mechanisms may be involved in the regulation of pancreatic islet hormone secretion, independent of the classical dual autonomic innervation, although this assumes that the peptidergic system is unaffected by atropine.

A marked elevation of plasma growth hormone levels was observed in both groups of tetraplegic subjects after hypoglycaemia. However, a normal glucose recovery has been found in the absence of a rise of growth hormone (Greenwood and Landon, 1966; Abrams et al., 1966; Feldman et al., 1975), and studies in man using somatostatin infusions have indicated that growth hormone is not essential for blood glucose homeostasis (Gerich et al., 1979; Rizza et al., 1979a). The release

of growth hormone in response to hypoglycaemia is decreased by alpha-receptor blockade (Blackard and Heidingsfelder, 1968; Clarke et al., 1979), and stimulation of growth hormone may be mediated at least in part via an alpha-adrenergic receptor mechanism. The increased GH response in the sympathectomised subjects in response to hypoglycaemia in the present study is consistent with the increased GH response in normal subjects under beta blockade with propranolol (Abramson et al., 1966) and during glucopenia induced with 2-deoxy-D-glucose in sympathectomised (Brodows et al., 1973) and adrenalectomised patients (Brodows et al., 1975). These findings suggest that the overall effect of adrenergic activation during hypoglycaemia is to inhibit GH secretion via a beta receptor mechanism.

The delayed rise of plasma ACTH, and the reduced plasma cortisol response in the tetraplegic patients under cholinergic blockade, were inappropriate to the severe hypoglycaemia and may have been responsible for the impaired blood glucose recovery in these subjects, despite the adequate secretion of pancreatic glucagon and growth hormone. Cortisol is thought to encourage gluconeogenesis by a permissive effect on the actions of glucagon and catecholamines to stimulate hepatic glycogenolysis and gluconeogenesis (Exton et al., 1972) and also by its influence upon catecholamine-induced release of lactate from skeletal muscle and glycerol from adipose tissue (Steele, 1975). Furthermore, synergistic interactions of glucagon, adrenaline and cortisol to produce hyperglycaemia have been demonstrated in the dog (Eigler et al., 1979). In these animals when all three hormones were infused simultaneously, the increment in blood glucose was two to fourfold greater than the sum of the responses of the individual hormone infusions. The infusion of cortisol alone had no effect on glucose production, but it markedly accentuated the hyperglycaemia

produced by glucagon and/or adrenaline. Thus, physiological elevations in circulating cortisol may not have merely a permissive role on the activity of the other counterregulatory hormones, but may prolong their actions on the liver (Eigler et al., 1979).

Impaired blood glucose recovery from hypoglycaemia has been described in states of primary and secondary adrenal insufficiency (Fraser et al., 1941; De Bodo and Altszuler, 1958; Shahmanesh et al., 1980), although diminished blood glucose recovery was not apparent when replacement corticosteroid therapy was given prior to the induction of hypoglycaemia (Ginsburg and Paton, 1956; Ensinck et al., 1976; Brodows et al., 1976). In tetraplegic subjects in whom the catecholamine response is absent, a relative deficiency of cortisol following hypoglycaemia may cause impairment of blood glucose recovery, in view of its permissive and synergistic interactions with glucagon. This may occur particularly if the availability of lactate for gluconeogenesis is limited, as demonstrated in the present studies (Chapter 7).

A cholinergic mechanism has been implicated in the secretion of corticotrophin-releasing factor in the murine hypothalamus (Jones et al., 1976). The present data suggest that a similar mechanism may exist in man; atropine crosses the blood-brain barrier (Innes and Nickerson, 1975) and could have delayed the activation of ACTH secretion in the tetraplegic group under cholinergic blockade.

Recovery from hypoglycaemia requires an integrated activation of various counterregulatory mechanisms which provide substrates for gluconeogenesis and stimulate hepatic glycogenolysis and gluconeogenesis. The present studies indicate that in the absence of peripheral adrenergic mechanisms alone this homeostatic process is relatively intact.

With the administration of atropine, both classical components of the autonomic nervous system are removed and impaired blood glucose recovery becomes manifest. This impairment of the recovery mechanism may be explained by a deficiency of circulating cortisol secondary to the blockade of central cholinergic receptors involved in the activation of ACTH secretion. The present studies do not support a direct role for peripheral cholinergic receptors in the activation of pancreatic glucagon secretion in response to hypoglycaemia.

APPENDIX

ACTH assay (Ratcliffe, J.G. and Gray, C.E., unpublished). This is a double antibody radioimmunoassay on unextracted plasma:

200 µl plasma (in duplicate) - thawed immediately before assay
200 µl diluent in acidic buffer
100 µl Rabbit Anti-ACTH Antiserum (Medi-lab; Denmark)
final dilution 1 : 48,000 in EDTA buffer
100 µl ^{125}I-human ACTH 25 pg (Dr P.J. Lowry) in acidic buffer
(prepared by chloramine-T method)

Incubate overnight at 4°C
Double antibody (Donkey anti-rabbit) precipitation overnight at 4°C
Centrifuge and count pellet

Standardisation against WHO Standard 74/555
Standard curve covers range ~10 - 20 ng/l to 640 ng/l
High samples diluted in phosphate albumin buffer
Precision (between batch cv) ~10 - 20%

The assay correlates well in general with the previous extracted ACTH assay though there may be some discrepant situations since the unextracted assays probably cross-react with high molecular weight components to a greater extent than the extracted.

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PUBLISHED WORK RELATED TO THIS THESIS

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ABSTRACTS

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Suppression of endogenous insulin secretion after acute hypoglycaemia in man. By R. J. M. Corrall, B. M. Frier, D. Crothers and Joyce D. Baird. *Metabolic Unit, University Department of Medicine, Western General Hospital, Edinburgh, EH4 2XU*

Insulin and C-peptide are released in equimolar concentrations by the pancreatic beta cell. The development of a radioimmunoassay for plasma C-peptide reactivity (CPR; Heding, 1975) allows the endogenous secretion of insulin to be measured in the presence of exogenous insulin.

The response of the pancreatic beta cell to insulin-induced hypoglycaemia was studied in 11 normal young adults. Blood glucose and plasma CPR were measured during fasting, and at intervals for 180 min after the onset of the acute hypoglycaemic reaction induced by i.v. injection of insulin (0·15 units/kg body weight). Nine of these subjects were then given a standard mixed meal and blood sampling was continued for a further 120 min. The same meal was given to the nine subjects after fasting for an identical period without insulin administration and blood glucose and plasma CPR levels were measured as before.

After injection of insulin the fasting blood glucose level fell from $3{\cdot}93 \pm 0{\cdot}08$ (S.E.M.) to $1{\cdot}15 \pm 0{\cdot}08$ mmol/l within 20–30 min. Thereafter, in all subjects, the blood glucose concentration rose to regain the fasting level by 120 min after the onset of the acute hypoglycaemic reaction. After the meal, the mean blood glucose concentration rose to a peak of $8{\cdot}42 \pm 0{\cdot}27$ mmol/l at 60 min and was still raised at $7{\cdot}48 \pm 0{\cdot}29$ mmol/l at 120 min. In contrast, after fasting without hypoglycaemia, the mean blood glucose of the same subjects rose only slightly from $3{\cdot}82 \pm 0{\cdot}11$ to $5{\cdot}14 \pm 0{\cdot}24$ mmol/l at 30 min after the meal and returned to the fasting level by 60 min postprandially. The difference in the mean blood glucose concentration at 30, 60 and 120 min after the meal between both groups was highly significant ($P < 0{\cdot}001$).

In all subjects, plasma CPR fell rapidly after administration of insulin from a mean basal fasting level of $0{\cdot}39 \pm 0{\cdot}04$ pmol/ml to levels below the effective detection limit of the assay (0·06 pmol/ml) at 30 min after the onset of acute hypoglycaemic reaction. Secretion of CPR was completely suppressed until the reaction +150 min and after the reaction +180 min the mean CPR level was only $0{\cdot}11 \pm 0{\cdot}03$ pmol/ml. Levels of CPR rose after the meal with mean values of $0{\cdot}76 \pm 0{\cdot}15$ at 30 min, $1{\cdot}72 \pm 0{\cdot}24$ at 60 min and $2{\cdot}58 \pm 0{\cdot}47$ pmol/ml at 120 min. In contrast, when the meal was given after fasting alone, mean plasma CPR rose from $0{\cdot}35 \pm 0{\cdot}01$ to $1{\cdot}76 \pm 0{\cdot}08$ at 30 min, $1{\cdot}65 \pm 0{\cdot}18$ at 60 min and fell to $1{\cdot}16 \pm 0{\cdot}14$ pmol/ml at 120 min. Mean CPR values at 30 and 120 min after the meal were significantly different between the groups ($P < 0{\cdot}001$).

We conclude that after insulin-induced hypoglycaemia the normal response of the beta cell to a meal is markedly altered. Acute hypoglycaemia induces prolonged suppression of endogenous insulin secretion which continues after ingestion of food. Insulin secretion is sufficiently delayed to produce impaired carbohydrate tolerance.

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Journal of Endocrinology, 81, 157P, (1979)

EFFECT OF PREGANGLIONIC SYMPATHECTOMY ON METABOLIC RECOVERY FROM HYPOGLYCAEMIA

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To evaluate the role of the sympathetic nervous system in the recovery from acute hypoglycaemia, six male subjects with complete post-traumatic transection of the cervical spinal cord were studied, and the results compared with 11 normal controls. Insulin (0·15 unit/kg) was administered intravenously to fasting subjects and sequential blood samples were assayed. The recovery of blood glucose after hypoglycaemia was unimpaired in the sympathectomized subjects, although a rise in blood lactate concentration was not observed in this group. Changes in plasma free fatty acid and glucagon concentrations were similar in both groups. The marked increase in plasma cyclic-AMP and plasma noradrenaline concentrations seen in normal subjects was absent in the sympathectomized group.

This study demonstrates that, after a total preganglionic sympathectomy in man, blood glucose recovery from insulin-induced hypoglycaemia is normal. Glucagon secretion appears to be intact, but a rise in plasma cyclic-AMP concentrations does not occur. The putative role of adrenergic mechanisms in the homeostatic recovery from hypoglycaemia in man may require re-evaluation.

Clinical Science, 57, 24P, (1979)

Attenuation of beta-cell function following acute hypoglycaemia in man

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The response of the pancreatic beta-cell to hypoglycaemia was studied in 11 normal young adults (hypoglycaemia study). Blood glucose and plasma C-peptide reactivity (CPR) were measured fasting and at intervals for 210 min after the administration of i.v. insulin (0.15 units/kg body weight). Nine subjects were then given a standard mixed meal and blood sampling was continued for a further 120 min. The same meal was given to the 9 subjects after fasting for an identical period without insulin being administered (fasting study). A normal recovery pattern of blood glucose was observed following hypoglycaemia, and C-peptide secretion was completely suppressed until 180 min after the injection of insulin. After the meal, mean blood glucose in the hypoglycaemia study rose to a significantly higher level than in the fasting study. After hypoglycaemia, CPR secretion was much slower following the meal, compared to the fasting group, but significantly higher plasma CPR levels were subsequently achieved by 120 min postprandially. This abnormal pattern of insulin secretion after the meal resembles the defect observed in maturity-onset diabetes. The possible mechanisms of this phenomenon are discussed.

Excerpta Medica, 481, 68, (1979)

Control of pancreatic islet cell function in man: investigation of adrenergic and cholinergic mechanisms. By B. M. Frier, R. J. M. Corrall, J. P. Ashby, E. J. W. McClemont* and D. Shirling. *Metabolic Unit, University Department of Medicine, Western General Hospital, Edinburgh, EH4 2XU, and *Spinal Unit, Edenhall Hospital, Musselburgh, East Lothian*

The pancreatic islets receive a rich cholinergic and adrenergic innervation. We have examined the role of the neural control of the pancreatic α and β cells in response to hypoglycaemia in man.

Insulin (0·15 u./kg) was given i.v. to 11 normal subjects, six tetraplegic patients with complete transection of the cervical spinal cord (pre-ganglionic sympathectomy alone) and six tetraplegic patients under cholinergic blockade with i.v. atropine (total autonomic denervation of pancreas). Levels of blood glucose, plasma insulin, C-peptide (CPR), glucagon and noradrenaline were measured in the basal state and up to 210 min after the injection of insulin.

Blood-glucose recovery from hypoglycaemia was impaired only in the tetraplegic patients given atropine. Plasma CPR fell during hypoglycaemia and remained low despite restoration of euglycaemia. Plasma glucagon increased in all three groups; the highest levels were observed in the subjects with total autonomic denervation. In all tetraplegic subjects, there was no rise in plasma noradrenaline in response to hypoglycaemia.

The impaired blood-glucose recovery associated with total autonomic denervation cannot be explained by an abnormal secretion of glucagon. This study suggested that, in man, the response of the pancreatic islets to hypoglycaemia may occur in the absence of control through cholinergic and adrenergic mechanisms.

Journal of Endocrinology, 85, 30P - 31P, (1980)

ABNORMAL INSULIN SECRETORY RESPONSE TO FOOD AFTER ACUTE HYPOGLYCAEMIA IN MAN: EVIDENCE FOR β-CELL GLUCOPENIA

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Abnormal function of the pancreatic β-cell and impaired carbohydrate tolerance have been observed in response to a meal after actue hypoglycaemia in man (Corrall, Frier, Crothers & Baird, 1979, *Journal of Endocrinology*, **81**, 157P). This was characterized by an early reduced postprandial rise in plasma insulin and abnormally elevated levels 2 h later. This temporary disturbance of insulin secretion was investigated as follows. (1) Gastrointestinal hormones: serial levels of enteroglucagon, motilin, neurotensin, gastric inhibitory peptide, gastrin and pancreatic polypeptide were assayed in six normal subjects during hypoglycaemia (0·15 unit of insulin/kg, intravenously) and in response to a subsequent meal. (2) Glucose infusion: after hypoglycaemia, glucose (0·5 g/kg) was infused intravenously over 3 min in four normal subjects at 60 min before the meal, to reverse possible β-cell glucopenia. (3) Adrenergic inhibition: the insulin secretory response to a meal after hypoglycaemia was studied in two subjects with complete preganglionic sympathectomy. (4) Aminophylline infusion: this was performed to investigate a possible role of intracellular cyclic AMP (cAMP) depletion in causing abnormal β-cell function. Aminophylline (250 mg intravenously and 4 mg/min for 60 min) was administered 60 min before the meal after hypoglycaemia in two normal subjects.

The results do not support mediation via abnormal gastrointestinal hormone secretion (entero–insular axis), adrenergic inhibition or intracellular cAMP deficiency of the pancreatic β cell. A partial improvement of the abnormal postprandial pattern of insulin secretion by a preceding glucose infusion suggests that glucopenia of the β cell is an important factor in the pathogenesis of this abnormality.

Clinical Science, 59, 16P, (1980)

16. Neuroendocrine Counterregulatory Mechanisms in the Recovery from Acute Hypoglycaemia in Man

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The recovery from acute insulin-induced hypoglycaemia (IV insulin 0.15 U/kg) was studied in 11 normal subjects, 6 subjects with preganglionic sympathectomy (adrenergic denervation) and 6 sympathectomised subjects given atropine (combined adrenergic denervation and cholinergic blockade). Blood glucose recovery was impaired only in the sympathectomised subjects given atropine. The blood lactate response was reduced and the rise in non-esterified fatty acids was delayed in both groups of sympathectomised subjects, in whom the normal rises of plasma cyclic AMP and noradrenaline were absent. The changes in plasma C-peptide and glucagon levels were appropriate to prevailing blood glucose concentrations in all 3 groups. Cortisol and ACTH responses were impaired in the group of sympathectomised subjects given atropine and growth hormone levels were higher in both sympathectomised groups. – Blood glucose homeostasis was clearly impaired during combined adrenergic and cholinergic denervation. Under such conditions appropriate islet cell responses were retained; glucagon secretion was activated independent of vagal control. In the sympathectomised group given atropine the rise in plasma cortisol was inappropriately blunted in response to a greater degree of hypoglycaemia. Impaired adrenocortical activation at hypothalamic level may explain, at least in part, the delayed restoration of normoglycaemia.

Diabetologia, 19, 560 (1980)

Acute Hypoglycemia in Man: Neural Control of Pancreatic Islet Cell Function

Roger J. M. Corrall and Brian M. Frier

Acute hypoglycemia is associated with stimulation of the pancreatic alpha cells and a concurrent, prolonged suppression of insulin secretion by the beta cells. The islets receive a rich autonomic innervation and may therefore be subject to control by adrenergic and cholinergic mechanisms. The role of such neuroregulation of pancreatic islets in response to hypoglycemia has been examined in normal subjects, in subjects with a preganglionic sympathectomy due to traumatic tetraplegia, and in tetraplegic subjects given atropine to induce effective dual autonomic denervation. The normal rise of plasma norepinephrine following hypoglycemia was absent in both groups of tetraplegic subjects, providing evidence of a complete sympathectomy. Blood glucose recovery was significantly impaired only in the group of tetraplegic patients given atropine. Changes in plasma C-terminal glucagon-like immunoreactivity (C-GLI) and C-peptide immunoreactivity (CPR) following hypoglycemia were commensurate with blood glucose levels and were not significantly influenced by islet denervation. These observations suggest that neuroregulation of human islet cell function in response to hypoglycemia may be of limited importance and that stimulation of glucagon secretion may occur independent of cholinergic vagal control.

ACUTE HYPOGLYCEMIA in man induces an increase in the circulating levels of several hormones, including pancreatic glucagon.[1] In conjunction with this increase in alpha cell activity, there is a concomitant decrease in beta cell secretion of insulin, reflected by a reduction in plasma C-peptide concentrations.[2-4] The secretion of these two hormones, which have a central role in the regulation of carbohydrate homeostasis, may be influenced by many factors, including sympathetic adrenergic and parasympathetic cholinergic neural activity, and by circulating catecholamines. The relative contribution of these mechanisms to the control of pancreatic islet cell secretion during recovery from acute hypoglycemia is not clearly defined. To examine the importance of neural regulation, we have studied the pancreatic endocrine response to insulin-induced hypoglycemia in normal human subjects, and in subjects with adrenergic denervation of the pancreas, with and without cholinergic blockade.

MATERIALS AND METHODS

Subjects

Informed consent and Medical Ethics Committee approval was obtained for studies in three groups of subjects: Group A: Eleven normal, healthy subjects, (9 male, 2 female), age range 20–29 yr. All were within 10% of their ideal body weight (mean 96%, range 91%–102%). Group B: Six male subjects with complete post-traumatic transsection of the cervical spinal cord above C7, producing tetraplegia at least 6 mo before the study (duration of tetraplegia, 6 mo to 19 yr), age range 21–44 yr. All were within 10% of their ideal body weight (mean 94%, range 90%–98%). Group C: Six male tetraplegic subjects (2 from group B and 4 others), with similar post-traumatic transsection of the cervical spinal cord (duration 4 mo to 5 yr), age range 19–28 yr. All were within 10% of their ideal body weight (mean 97%, range 92%–104%). None of the subjects had a family history of diabetes mellitus.

Protocol

All subjects were studied supine after an overnight fast without preceding dietary preparation, and basal venous blood samples were taken for 30 min through a teflon cannula situated in an arm vein. Crystalline beef insulin, 0.15 U/kg body weight, was administered as a bolus by intravenous (i.v.) injection. The subjects in group C were given atropine, 15 μg/kg body weight, which was administered 30 min before the insulin. Effective atropinization was assessed by serial measurements of heart rate at 2-min intervals, and if necessary, atropine administration was repeated to maintain cholinergic blockade. Serial blood samples were withdrawn at 30-min intervals for up to 210 min after the administration of insulin, and were stored at −20°C before being assayed with minimal delay for blood glucose,[5] plasma immunoreactive insulin (IRI),[6] C-peptide immunoreactivity (CPR),[7] C-terminal reactive glucagon-like immunoreactivity (C-GLI),[8] and plasma norepinephrine concentrations.[9]

Statistical significance was estimated using the unpaired Student's t test.

RESULTS

The normal subjects experienced a typical hypoglycemic reaction at approximately 30 min after the injection of insulin, characterized by visible sweating, tachycardia, and symptoms of neuroglycopenia. In group B, a tachycardia was absent and there was no visible sweating, but mild neuroglycopenia was experienced. The tetraplegic subjects lack a normal sympathetic cardiac innervation and in group C the adequacy of cholinergic blockade was confirmed by a sustained tachycardia following the administration of atropine. The pulse rate rose from a mean basal rate of 61 beats/minute (range 36–72) to 89 beats/minute (range 64–100), and in 3 subjects a second dose of atropine was given between 100 and 135 min after the initial atropine injection to maintain the tachycardia.

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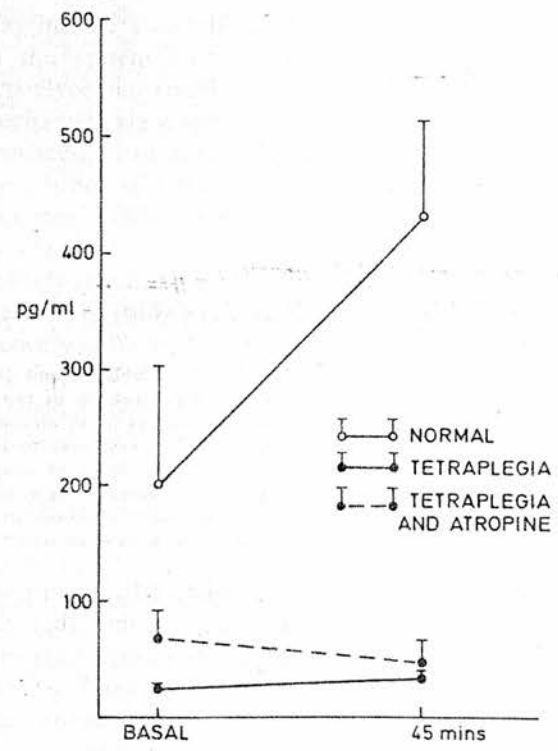

Fig. 1. Plasma norepinephrine concentrations (mean ± SEM) in normal and tetraplegic subjects in the basal state and 45 min after insulin administration.

In group C, no visible sweating was observed during hypoglycemia but the neuroglycopenic symptoms were more severe and prolonged. In two of the patients who had received atropine the study had to be terminated with parenteral glucose 90 min after the injection of insulin, because of severe symptoms of neuroglycopenia.

Following the injection of insulin, plasma IRI rose to similar peak levels with a subsequent exponential fall in all three groups of subjects. In the normal subjects the mean norepinephrine concentration rose to double the basal value at 45 min after insulin administration (Fig. 1). In the tetraplegic patients (groups B and C) the basal concentrations of norepinephrine were much lower, and no significant change was observed following hypoglycemia.

Figure 2 depicts the changes in mean blood glucose concentration in all three groups. In both groups of tetraplegic subjects, the fall in blood glucose after insulin administration was slower than in the normal subjects, and a lesser degree of hypoglycemia was attained. At 30 min the mean blood glucose in the normal group was significantly lower than that recorded in the tetraplegic group without atropine ($p < 0.001$), and the tetraplegic group with atropine ($p < 0.001$). There was a significant difference at this time in mean blood glucose between the two groups of

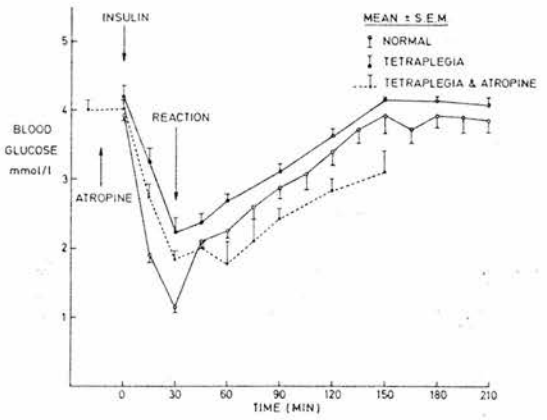

Fig. 2. Blood glucose concentrations (mean ± SEM) in normal and tetraplegic subjects in response to i.v. insulin. In the normal subjects "REACTION" denotes the onset of the acute tachycardia and the symptoms of hypoglycemia.

tetraplegic subjects ($p < 0.05$). In group B blood glucose recovery proceeded at a similar rate to the normal subjects. The small difference in blood glucose between these two groups did not achieve statistical significance during the recovery phase. In contrast the recovery from hypoglycemia in the tetraplegic patients given atropine (group C) was much slower than in the other two groups, indicating a significant impairment of blood glucose recovery during combined adrenergic denervation and pharmacologic cholinergic blockage. Between groups A and C there was a significant difference in the mean blood glucose levels at 60 min ($p < 0.01$), 90 min ($p < 0.001$) and at 150 min ($p < 0.001$). The individual blood glucose values for those tetraplegic subjects given atropine are shown in Fig. 3.

Mean plasma CPR concentrations are shown in Fig. 4. The mean basal CPR levels were significantly higher in both groups of tetraplegic patients compared to the normal subjects (group B, $p < 0.001$; group C $p < 0.05$), but the mean basal values of groups B and C were not significantly different from each other. Mean plasma CPR concentration fell following the injection of insulin in all three groups. In the normal subjects the mean plasma CPR levels decreased to the effective detection limit of the assay and had not returned to the normal basal level by the end of the study, despite the restoration of euglycemia. A similar pattern was observed in the tetraplegic subjects without atropine, but the mean plasma CPR concentrations were significantly higher than in the normal subjects ($p < 0.001$) from 60 min onwards, consistent with the lesser degree of hypoglycemia attained in this group. An intermediate response was observed in the tetraplegic group given atropine, with a decline in the mean plasma CPR level to the effective detection limit of the assay at 150 min (Fig. 4).

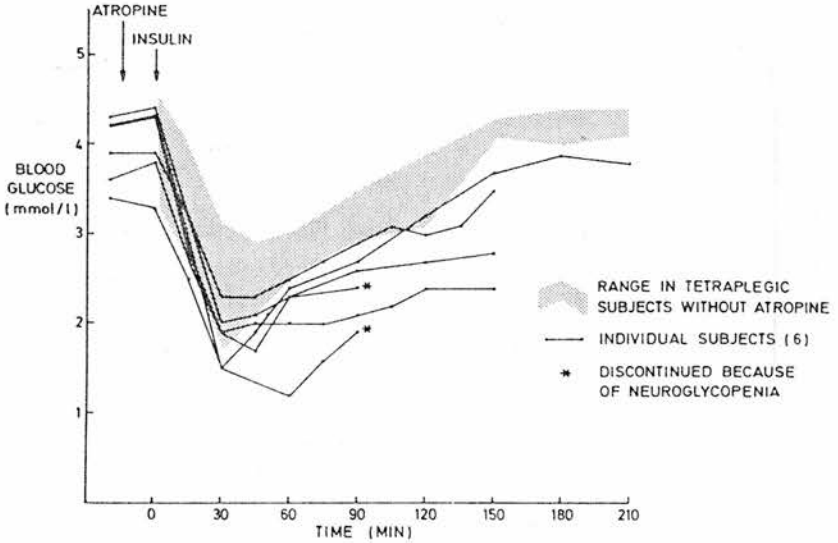

Fig. 3. Individual blood glucose concentrations in tetraplegic subjects given atropine (group C) in response to i.v. insulin. The range of blood glucose concentrations in tetraplegic subjects without atropine (group B) is illustrated.

The mean plasma C-GLI concentrations are depicted in Fig. 5. The mean basal plasma C-GLI level was lower in the normal group but this difference compared to either tetraplegic group failed to achieve statistical significance. Plasma C-GLI rose in response to hypoglycemia in groups A and B with peak values at 60 min. In group C plasma C-GLI also rose, with prolongation of the secretory response; higher concentrations were observed at 60 and 90 min which were statistically significant at 90 min in comparison with both group A, ($p < 0.05$) and group B, ($p < 0.02$). The percentage increments of mean glucagon values from basal to 60 min in the three groups of subjects (group A, 122%; group B, 41%; group C, 63%) were proportional to the percentage fall in mean blood glucose from basal to its nadir in the same groups (group A, 71%; group B, 47%; group C, 54%).

DISCUSSION

In this study we have examined in vivo the separate adrenergic and cholinergic mechanisms that may influence human pancreatic islet cell activity in response to hypoglycemia. It is possible that the administration of atropine in this dosage may not achieve complete cholinergic blockade of the pancreatic islets. However, the sustained tachycardia in the absence of sympathetic cardiac innervation supports a significant degree of cholinergic blockade following atropine in these subjects. Evidence for complete disruption of the efferent sympathetic pathway in these tetraplegic subjects is presented by the absence of sweating and tachycardia in response to hypoglycemia, and by the low basal norepinephrine levels which failed to rise following hypoglycemia, as found by Palmer et al.[10] Ablative studies in animals have suggested the existence of subhypothalamic centers in the cervical and thoracic spinal cord that

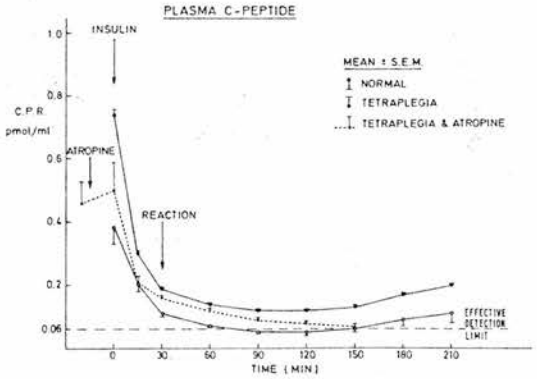

Fig. 4. Plasma CPR (mean ± SEM) in normal and tetraplegic subjects in response to i.v. insulin.

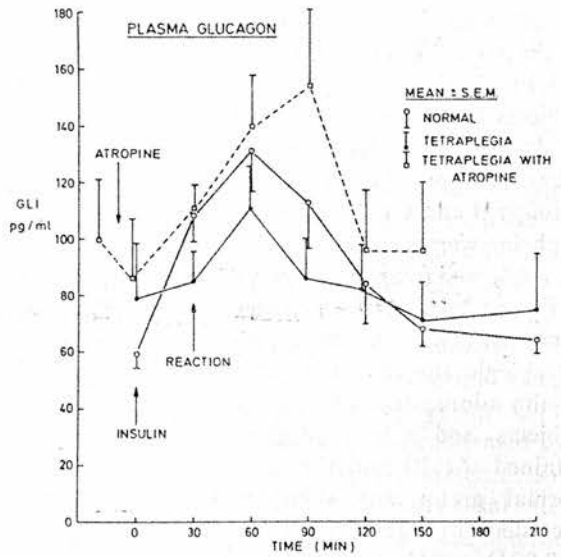

Fig. 5. Plasma C-GLI concentrations (mean ± SEM) in normal and tetraplegic subjects in response to i.v. insulin.

may initiate a metabolic response to hypoglycemia.[11] In the present study, it is therefore possible that hypoglycemia could activate a counter-regulatory mechanism via a spinal cord reflex below the level of transsection. In human tetraplegic subjects however, the infusion of 2-deoxy-glucose to induce intracellular glucopenia, did not induce a rise of blood glucose levels as observed in normal subjects.[12] It is therefore unlikely that a spinal reflex in man could be invoked in the present study to explain the normal blood glucose recovery in the tetraplegic subjects (group B).

Compared with the normal subjects, the slower fall in blood glucose and the lesser degree of hypoglycemia achieved in the tetraplegic subjects without atropine, suggests a relative resistance to insulin, which may be related to their chronic inactivity.[13] Palmer et al. attempted to circumvent this relative insensitivity to insulin by administering a larger dose (0.2 U/kg body weight) to their tetraplegic patients, but still did not achieve equivalent hypoglycemia.[10] In the present study, all subjects received the same dose of insulin (0.15 U/kg), but in the tetraplegic subjects under cholinergic blockade (group C), blood glucose fell lower, and recovery from hypoglycemia was significantly delayed compared with group B, which necessitated discontinuation of the study in two patients. This impairment of blood glucose recovery cannot be explained by impaired secretion of pancreatic glucagon or by a failure of the normal prolonged inhibition of insulin secretion which accompanies the restoration of euglycemia.[2,4] Further studies are being undertaken to determine the cause of this impairment of the homeostatic recovery mechanism.

Langerhans observed that the pancreatic islets receive an extensive innervation.[14] Fluorescent and histochemical techniques have shown the presence of both adrenergic and cholinergic nerve fibres supplying the islet secretory cells.[15] Animal studies have demonstrated that stimulation of the parasympathetic nervous system promotes insulin secretion, whereas beta cell activity is inhibited by sympathetic nerve stimulation and by catecholamines acting via an alpha-adrenergic receptor mechanism.[15] In man, activation of the sympathetic nervous system occurs in response to hypoglycemia producing a marked rise in plasma and urinary catecholamines.[1,16–18] The decline in plasma C-peptide concentration following hypoglycemia was observed in both groups of tetraplegic subjects, and was commensurate with the changes in blood glucose. It is apparent therefore that pancreatic beta cell suppression in response to hypoglycemia may occur in man even in the absence of normal sympathetic innervation and concomitant pharmacologic cholinergic blockade.

Several studies have shown that glucagon secretion is stimulated in vitro by adrenergic agonists[19–21] and in vivo by the infusion of catecholamines,[22] or by neural stimulation.[23–25] The work of Bloom et al.[26] in the calf suggested a cholinergic control of glucagon secretion via the vagus nerve, and in this species the secretion of glucagon in response to hypoglycemia was significantly delayed by atropine. The glucagon response to acute hypoglycemia was reduced in human subjects following truncal vagotomy, which was interpreted as evidence for a cholinergic control of alpha cell activity in man.[27] This was not confirmed by Palmer et al.[28] who showed no effect of vagotomy or cholinergic blockade on glucagon secretion in man in response to hypoglycemia.

The previous finding of normal glucagon release during hypoglycemia in patients with a preganglionic sympathectomy[10] and under combined alpha and beta adrenergic blockade,[29] is confirmed by this study, and suggests that the sympathetic nervous system has a limited involvement in modulating the secretion of pancreatic glucagon in response to hypoglycemia. In the present study glucagon secretion was unaffected by co-existing cholinergic blockade and was augmented during prolonged hypoglycemia. These findings contradict the contention that human pancreatic alpha cell function is largely controlled by vagal cholinergic activity, and are consistent with the findings of Palmer et al.[28] Alpha cells in tissue culture are stimulated to release glucagon by low glucose concentrations,[30] suggesting that hypoglycemia exerts a direct effect via glucopenia. The present study indicates that under specific conditions, appropriate islet cell responses may be observed despite adrenergic denervation and pharmacological blockade of cholinergic mechanisms of control.

Peptidergic neurons are contained within the vagus nerve and within the extensive plexus of nerve fibres in the pancreas.[31] Somatostatin-like immunoreactivity has been demonstrated in peripheral adrenergic neurons in the guinea-pig[32] and in the D cells of human pancreatic islets.[33] Furthermore, plasma somatostatin levels are persistently raised during the recovery from insulin-induced hypoglycemia in man.[34] Vasoactive intestinal peptide (VIP) has been found in fibres of the vagus nerve,[31,35] and is released in response to various physiologic stimuli, including direct electrical stimulation of the vagus nerve. VIP has also been shown to stimulate pancreatic glucagon secretion in the perfused cat pancreas.[36] It is possible therefore that peptidergic mechanisms may be involved in the regulation of pancreatic islet hormone secretion, independent of the classical dual autonomic innervation.

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The Mechanism of Abnormal Pancreatic Beta Cell Response to Food Following Acute Hypoglycaemia in Man

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Summary

Following acute insulin-induced hypoglycaemia, an abnormal pattern of insulin secretion in response to a meal has been demonstrated in six healthy volunteers. This is characterised by an initial impairment in insulin secretion and late hyperinsulinaemia. Postprandial gastrointestinal hormone levels were normal following hypoglycaemia in these subjects. In four other subjects, the administration of intravenous glucose prior to the meal partially reversed the abnormal pattern of secretion. In two patients with a total pre-ganglionic sympathectomy, the pattern of blood glucose, plasma insulin and C-peptide was similar to that observed following hypoglycaemia in normal subjects. It is unlikely that an abnormal entero-insular axis or an elevation of plasma catecholamine levels are primarily responsible for this phenomenon. This effect of hypoglycaemia on postprandial insulin secretion may be caused by glucopenia of the pancreatic beta cells.

Key-Words: *Hypoglycaemia – Insulin Secretion – Entero-Insular Axis – Beta Cell Glucopenia*

Introduction

Abnormal function of the pancreatic beta cell has been observed in response to a meal following acute hypoglycaemia in man (*Frier, Corrall, Ashby and Baird* 1980). In that study, following the ingestion of food, carbohydrate intolerance was associated with an abnormal pattern of insulin secretion, characterised by a subnormal early postprandial rise of plasma insulin and an abnormal elevation two hours later. Possible mechanisms underlying this altered pattern of postprandial insulin secretion include the following: (a) disordered function of the entero-insular axis following hypoglycaemia, (b) intrinsic pancreatic beta cell dysfunction caused by glucopenia and (c) inhibition of the beta cell by catecholamines released during hypoglycaemia. In the present study we have investigated the relative importance of these possible mechanisms.

Subjects and Methods

The following studies were approved by the Medical Ethics Committee and informed consent was given by the subjects, all of whom were within 10% of their ideal body weight. In all studies the subjects were investigated supine, after an overnight fast.

Protocol:

(a) *Hypoglycaemia study.* Crystalline beef insulin (0.15 units/kg body weight) was injected as an intravenous bolus to induce acute hypoglycaemia; this was experienced as typical neuroglycopenic and autonomic symptoms by all normal subjects after 20 to 30 minutes. Serial blood samples were taken in the fasting state, and for 210 minutes after the injection of insulin, followed by a standard mixed meal (30 G protein, 85 G carbohydrate and 40 G fat). Blood sampling was continued for a further 120 minutes after the meal.

(b) *Fasting Study.* The same subjects were restudied after fasting for an equivalent period without the administration of insulin and similar measurements were made after an identical meal.

The above protocol was used for the following investigations:

1. *Gastrointestinal hormones.* In six normal male subjects, age range 21–29 years, serial assays were made of plasma entero-glucagon (*Thomson and Bloom* 1976), motilin (*Bloom, Mitznegg and Bryant* 1976), neurotensin (*Blackburn and Bloom* 1979), gastrin (*Russell, Bloom, Fielding and Bryant* 1976), gastric inhibitory peptide (*Sarson, Bryant and Bloom* 1980) and pancreatic polypeptide (*Adrian, Bloom, Bryant, Polak, Heitz and Barnes* 1976), during both the hypoglycaemia and fasting studies. Cross-reactivity of the antisera raised to the various peptides assayed was less than 0.5 per cent. Antiserum R59, which showed a high degree of cross-reactivity with ileal extracts, and fully detected gravimetrically-determined glicentin, was used to measure total plasma glucagon. Enteroglucagon concentration was derived by subtraction of the pancreatic glucagon concentration measured by the pancreatic specific antiserum RCS5, from total plasma glucagon. Serial measurements were also made of blood glucose (*Hill and Kessler* 1961), plasma immunoreactive insulin (IRI) assayed against a human insulin standard, RD10, (*Ashby and Speake* 1975) and plasma C-peptide immunoreactivity (CPR) using an anti-synthetic human C-peptide guinea pig serum, M1230 (*Heding* 1975).

2. *Glucose infusion.* A possible effect of hypoglycaemia on pancreatic beta cell function was evaluated by the administration of intravenous glucose to four other normal subjects (3 male, 1 female; age range 22–24 years) using a modification of the above protocol. At 150 minutes after the injection of insulin, dextrose (0.5 G/kg body weight) was infused i.v. over three minutes in a total volume of 250 ml (*Soeldner* 1971) following hypoglycaemia, or after equivalent periods of fasting. Serial measurements were made of blood glucose, plasma IRI and plasma CPR. After the i.v. glucose load blood glucose was determined at 10 minute intervals for 60 minutes prior to the ingestion of food. In the fasting study an identical glucose infusion was given at an equivalent time prior to the meal.

3. *Catecholamines.* The effect of hypoglycaemia on insulin secretion in response to a meal was examined in two male subjects (aged 22 and 44 years) with traumatic transection of the cervical spinal cord above the sympathetic outflow (pre-ganglionic sympathectomy). Blood glucose and plasma IRI and CPR were measured.

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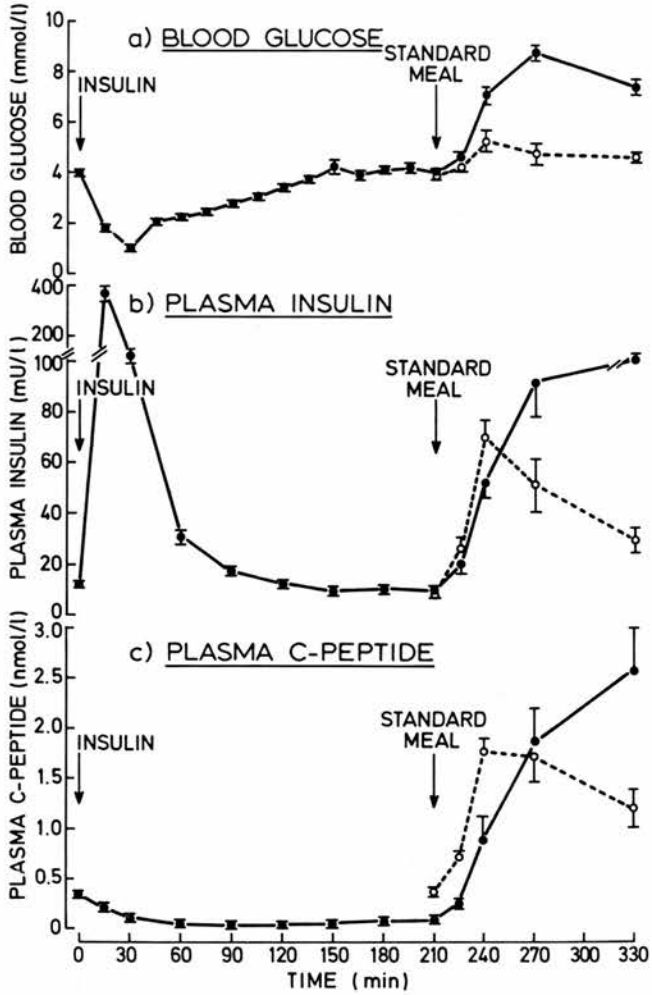

Fig. 1 Blood glucose, plasma insulin and C-peptide concentrations (Mean ± SEM) in 6 normal subjects, in response to hypoglycaemia followed by a standard meal (hypoglycaemia study ●—●), and in response to a standard meal without preceding hypoglycaemia (fasting study ○– –○)

Results

Following the injection of insulin the fall in blood glucose was followed by a typical recovery pattern to a normal mean fasting level by 150 minutes in the group of six normal subjects (Fig. 1). Following the injection of insulin, after an initial peak, the mean plasma IRI fell rapidly to a level within the normal fasting range. The mean plasma CPR concentration declined after insulin injection, and remained persistently depressed below the normal fasting level until the meal. Following the meal, postprandial hyperglycaemia was associated with early subnormal insulin and C-peptide secretion, then subsequent hypersecretion in comparison with the fasting study (Fig. 1).

G.I. Hormones. The pattern of secretion of individual gastrointestinal hormones in the same six subjects during the hypoglycaemia and fasting studies is shown in Fig. 2. A marked increase in pancreatic polypeptide was observed during hypoglycaemia, but secretion of the other G.I. hormones was not significantly changed. The secretion patterns of all these hormones in response to the meal were not significantly different between the hypoglycaemia and fasting studies.

Glucose infusion. The changes in mean blood glucose concentration in the four subjects given i.v. glucose 60 minutes before the meal are illustrated in Fig. 3. Following the i.v. infusion of glucose the peak blood glucose level was comparable in both the hypoglycaemia and fasting studies, but the mean level then fell to a significantly lower level in the fasting study at 50 and 60 minutes after the injection of glucose ($P < 0.05$). Following the meal, the blood glucose levels were significantly higher after hypoglycaemia in comparison with the fasting study, at all times of measurement ($P < 0.001$). However, the incremental rise from the different preprandial mean blood glucose levels and the subsequent pattern of glucose tolerance were similar in both studies. This differs markedly from the postprandial blood glucose levels observed after hypoglycaemia alone (Fig. 3: shaded area).

Plasma IRI and CPR concentrations both rose in response to the infusion of glucose, and in individual subjects the increments of both plasma IRI and CPR were smaller following hypoglycaemia. The mean increment of plasma IRI in response to this infusion was 23.5 ± 7.2 mU/l in the hypoglycaemia study, compared to 36.8 ± 11.7 mU/l in the fasting study; statistical significance was not achieved in this small group of subjects. In response to the meal (preceded by parenteral glucose) the pattern of insulin secretion was similar in both the hypoglycaemia and the fasting studies, but significantly higher levels of plasma IRI and CPR were observed in the subjects following hypoglycaemia at 90 and 120 minutes after the meal ($P < 0.001$). Following hypoglycaemia plus a glucose infusion the rises in plasma IRI and CPR were more rapid during the first 15 minutes after the meal in comparison with the equivalent rise after hypoglycaemia alone.

Catecholamines. The pattern of blood glucose, plasma IRI and CPR observed in two subjects with preganglionic sympathectomy, in response to a meal following hypoglycaemia, is shown in Fig. 4. In comparison with normal fasting subjects postprandial hyperglycaemia was again present, and was accompanied by a later hypersecretion of insulin and C-peptide. The pattern in these two subjects was similar to that observed in normal subjects after hypoglycaemia.

Discussion

It is apparent that insulin-induced hypoglycaemia has a marked effect on insulin secretion by the pancreatic beta cell during the recovery from hypoglycaemia (*Horwitz, Rubenstein, Reynolds, Molnar and Yanaihara* 1975; *Service, Horwitz, Rubenstein, Kuzuya, Mako, Reynolds and Molnar* 1977; *Frier* et al. 1980). An impaired tolerance to oral glucose after hypoglycaemia was described by *Somogyi* (1951) and has been demonstrated following ingestion of a mixed meal (*Frier* et al. 1980). The present study confirms these observations, and suggests that the abnormal secretion of insulin following hypoglycaemia is not caused by a reduced secretion of gastrointestinal hormones

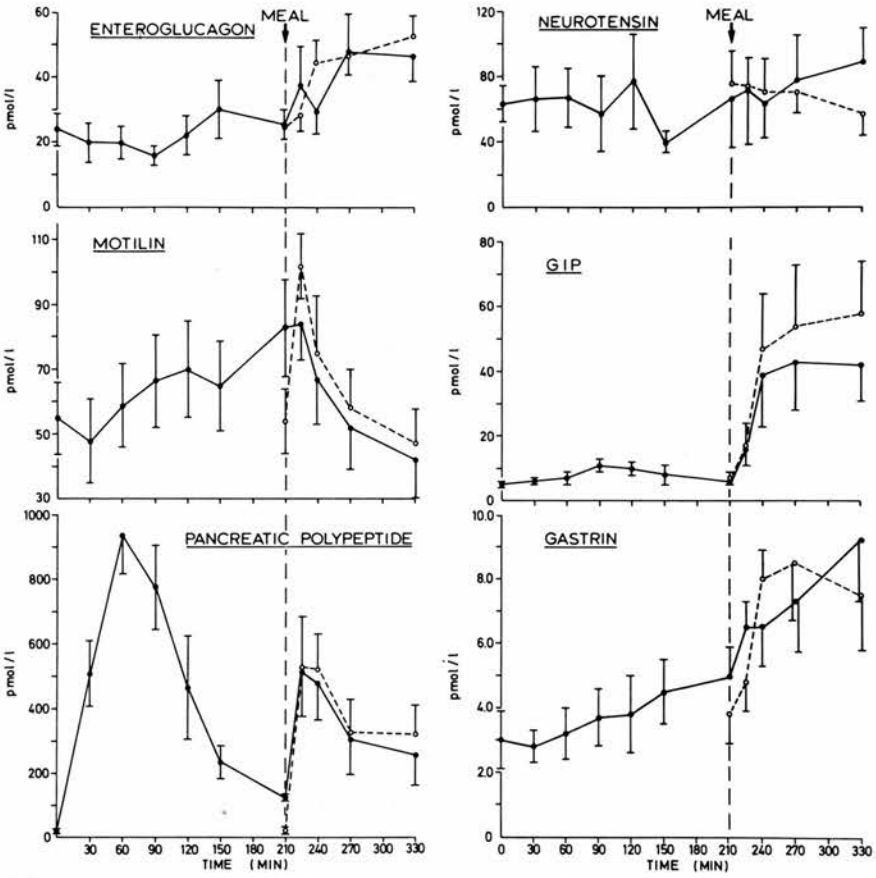

Fig. 2 Plasma concentrations (Mean ± SEM) of gastrointestinal hormones in normal subjects (n = 6) in the hypoglycaemia (●——●) and fasting (○— —○) studies

in response to a meal. The impaired tolerance to parenteral glucose following hypoglycaemia is also consistent with a mechanism that does not involve an abnormal enteroinsular axis.

The reduction in insulin secretion in response to intravenous glucose following hypoglycaemia suggests that beta cell function may be directly affected by preceding hypoglycaemia. The early subnormal secretion of glucose and the late hypersecretion of insulin, associated with glucose intolerance, was less marked. Thus the impaired capacity of the beta cell to respond to a meal following hypoglycaemia appeared to be partially reversed by a preceding intravenous infusion of glucose.

Intracellular cAMP has been implicated in the process of insulin secretion (*Sharp* 1979) and depletion of this nucleotide might explain the secretory pattern following hypoglycaemia. The administration of the phosphodiesterase inhibitor, aminophylline, has been reported to improve the deficient insulin response to a glucose infusion in patients with "prediabetes" (*Cerasi and Luft* 1969). In an attempt therefore to inhibit phosphodiesterase activity, aminophylline was administered to two of our normal subjects prior to the meal (250 mg i.v. followed by 4 mg/min for 60 minutes). This had no significant effect on the postprandial pattern of insulin secretion after hypoglycaemia, which suggests that the transient disturbance of beta cell function is not mediated by cAMP depletion.

A short infusion of adrenaline has been shown to produce a prolonged suppression of insulin secretion (*Robertson and Porte* 1973). We therefore investigated the possibility that a rise in plasma catecholamines during hypoglycaemia (*Garber, Cryer, Santiago, Haymond, Pagliara and Kipnis* 1976) might produce an abnormal pattern of insulin secretion in response to a subsequent meal. In the tetraplegic patients with a pre-ganglionic sympathectomy, plasma noradrenaline levels did not rise following hypoglycaemia (*Frier, Corrall, Ashby, McClemont and Sever* 1979). In these two subjects the postprandial pattern of insulin secretion was similar to that of normal subjects, suggesting that an adrenergic mechanism is not implicated.

The possibility that insulin may directly inhibit its own secretion has been studied by maintaining euglycaemia with a glucose clamp during the infusion of insulin (*Liljenquist, Horwitz, Jennings, Chiasson, Kellar and Rubenstein* 1978; *Service, Nelson, Rubenstein and Go* 1978). A partial suppression of plasma CPR concentrations was interpreted as evidence for such a direct negative feedback. In the present study, however, plasma insulin levels had returned to the normal fasting range prior to the ingestion of the meal. It appears unlikely therefore that such a feedback mechanism could explain the abnormal postprandial insulin secretion observed after acute hypoglycaemia, but it is not possible to prove that the initial high plasma insulin levels do not influence the pancreatic beta cells for several hours.

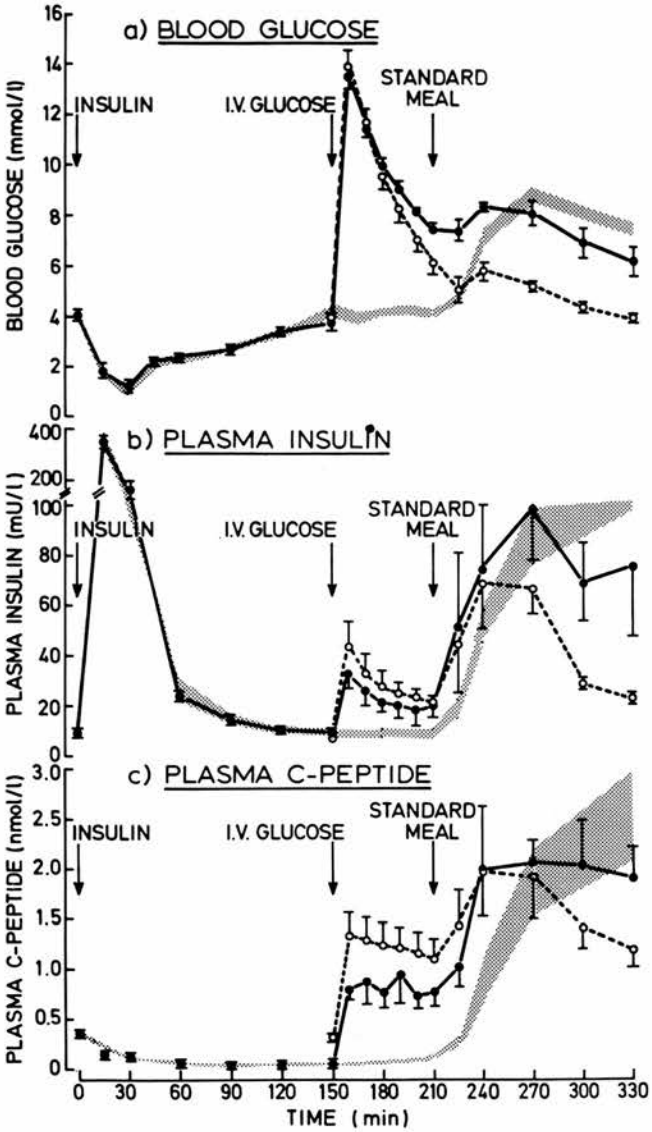

Fig. 3 I.V. glucose was administered 150 minutes after insulin injection to 4 normal subjects prior to a standard meal (hypoglycaemia study ●—●) or after an equivalent period of fasting (fasting study ○— —○). Blood glucose, plasma insulin and C-peptide concentrations are shown. The shaded area represents the mean ± SEM in normal subjects following insulin injection without i.v. glucose administration

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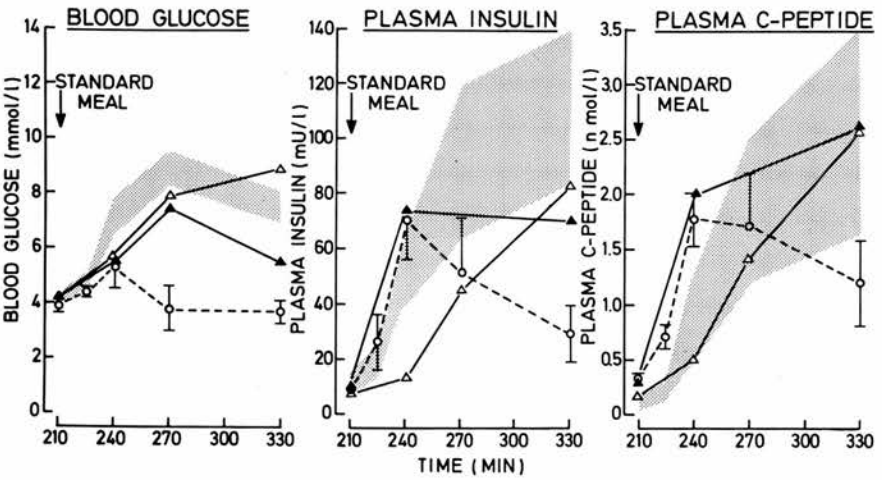

Fig. 4 Individual blood glucose, plasma insulin and C-peptide values in two subjects with a pre-ganglionic sympathectomy, in response to a standard meal following hypoglycaemia. (Time represented from 210 min after insulin injection). The shaded area represents the response (mean ± 2 SEM) in 6 normal subjects following hypoglycaemia in response to the same meal. The response to a meal in normal subjects after fasting is also shown (○— —○)

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Glucoregulatory Response to Intravenous Fructose Administration in the Dog

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Summary

This study was designed to evaluate the influence of fructose administration on glucose kinetics and the role of fructose conversion to glucose in the maintenance of glucose homeostasis. Intravenous fructose infusion (4.5 mg/kg min^{-1}) produced a stable plasma fructose concentration of about 20 mg/dl and a small but sustained decrease (10 mg/dl) of plasma glucose levels. The latter effect was entirely attributable to a rapid 30–35% fall in hepatic glucose output which later returned slowly to pre-infusion levels. No significant change in the rate of glucose utilization was observed. The rate of fructose conversion to glucose rose progressively during fructose infusion reaching a plateau of 1.4 mg/kg min^{-1} which corresponded to about 40% of total glucose production. Furthermore, as much as one third of the infused fructose was convertet to circulating glucose. No appreciable changes in plasma insulin and glucagon levels occurred during fructose infusion while plasma alanine concentration increased remarkably.

These data indicate that 1) fructose administration induces a transient fall in endogenous glucose production not accompanied by significant changes in glucose utilization; 2) the rapid conversion of the infused fructose to circulating glucose provides for the restoration of normal rates of glucose production; and 3) the glucoregulatory response to the administration of fructose occurs in the absence of detectable changes in plasma pancreatic hormone concentration.

Key-Words: *Fructose Conversion – Glucoregulatory Response – Dog*

Introduction

The existence of a close relationship between fructose and glucose metabolism *in vivo* is well recognized. Following intravenous administration, fructose is taken up by the liver and to a great extent, converted to glycogen (*Bergström, Fürst, Gallyas, Hultman, H:son Nilsson, Roch-Norlund and Vinnars* 1972; *H:son Nilsson and Hultman* 1974; *Miller, Craig, Drucker and Woodward* 1956; *Roch-Norlund, Hultman and H:son Nilsson* 1972). This process is very fast and appears to be independent of insulin availability (*Bergström, Fürst, Gallyas, Hultman, H:son Nilsson, Roch-Norlund and Vinnars* 1972; *Roch-Norlund, Hultman and H:son Nilsson* 1972). Little information is, however, available regarding the influence of fructose administration on glucose homeostasis. Human studies based on the hepatic vein catheter technique have suggested that splanchnic glucose output is inhibited during high dose i.v. fructose infusion (*Bergström and Hultman* 1967). Tracer studies have also indicated that fructose is a good glucogenetic substrate and that it may be converted to circulating glucose in significant amounts (*Atwell and Waterhouse* 1971; *Landau, Marshall, Craig, Hostetler and Genuth* 1971). The extent to which the conversion of infused fructose to plasma glucose may contribute to glucose production and the mechanisms whereby glucose homeostasis is maintained during the disposal of a fructose load remain, however, poorly understood. Furthermore, the question as to whether the administration of fructose modifies the rate of glucose utilization has not been explored.

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The present study was, therefore, undertaken to determine the effects of fructose administration on glucose kinetics in normal conscious dogs (i.e., hepatic glucose output and overall glucose utilization), and to quantitate the relative contribution of glucogenesis from fructose to total glucose production.

Materials and Methods

Experimental procedures. Experiments were performed on six male dogs (11–22 kg) which had been fed Purina dog chow for at least one week prior to study. All dogs were studied in the postabsorptive state after a 16 to 18-h overnight fast. On the morning of the study, a polyethylene catheter was inserted percutaneously into a saphenous vein for infusion of 3-^{3}H-glucose (Amersham) and fructose. Another catheter was similarly inserted into a femoral artery for blood sampling. A priming dose of tritiated glucose was administered rapidly (at −90 min) followed by a continuous tracer infusion at a rate of 100 nCi/min. The priming dose was 120-fold greater than the continuous infusion rate/min. A 90-min equilibration period was employed to insure that the plasma specific activity of 3-^{3}H-glucose had reached a stable plateau before measurement of changes in glucose kinetics. Then fructose was infused as a 40% solution at a rate of 4.5 mg/kg min^{-1} for 180 min. Twenty microcuries of ^{14}C-(U)-fructose (Amersham) were added to the solution of cold fructose so as to infuse approximately 80 nCi of labeled fructose per min.

Analyses. Plasma glucose concentration was measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Plasma fructose was determined by the method of *Heyrovsky* (1956). Alanine was measured by an enzymatic method using alanine dehydrogenase (*Williamson* 1974). The methods used for the determination of plasma immunoreactive insulin and glucagon (antibody 30K) have been previously described (*Saccà, Perez, Carteni and Rengo* 1977; *Saccà, Trimarco, Perez and Rengo* 1977). For the assay of 3-^{3}H-glucose radioactivity, plasma samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and the supernatant was evaporated to dryness at 70 °C to remove tritiated water. The dry residue was dissolved with 1 ml of water and counted with 10 ml of Insta-Gel (Packard Instruments, Downers Grove, Ill.). In order to quantitate fructose conversion to glucose it was necessary to determine the plasma specific activity of ^{14}C-fructose and ^{14}C-glucose. The specific activity of ^{14}C-fructose was assumed equal to the specific activity of the infused fructose since there is no endogenous production of this hexose. This assumption was verified experimentally as described below. For the determination of ^{14}C-glucose specific activity, the separation of this tracer from other labeled compounds was accomplished as follows. A two-ml aliquot of the Somogyi filtrate was passed through a first ion-exchange resin (Bio-Rad AG-2X8, 100-200 mesh) packed in a small column (0.4 x 5 cm) to remove acid components. Preliminary tests established that ^{14}C-glucose passes quantitatively through the column while the adsorption of ^{14}C-lactate and ^{14}C-pyruvate exceeded 98%. The effluent of the first column, containing mainly ^{14}C-glucose and ^{14}C-fructose, was then treated with glucose oxidase (20 U/ml) at 37 °C for 15 min in order to convert ^{14}C-glucose to ^{14}C-gluconic acid. The mixture was then applied to a second column containing the same resin (AG-2X8). Since the second chromatography was performed under the identical conditions of the first, only ^{14}C-gluconic acid was adsorbed while ^{14}C-fructose and other eventual labeled compounds not adsorbed by the first column also passed through the second column. ^{14}C-gluconic acid was then eluted with hydrochloric acid, collected into scintillation vials and mixed with Insta-Gel. The determination of radioactivity was carried out in a computerized Liquid Scintillation System (Tricarb 2660, Packard Instruments) which automatically corrected quench and spillover of ^{14}C in the tritium channel. In some experiments the difference in the counts of ^{14}C between the eluate of the first column (containing essentially ^{14}C-fructose and ^{14}C-glucose) and that of the second column (containing ^{14}C-gluconic acid) was used to estimate the specific activity of plasma ^{14}C-fructose. As expected, the values so obtained corresponded closely to the specific activity of the infused ^{14}C-fructose. The determination of ^{14}C-glucose specific activity by the current method is highly specific and accurate. The specificity is practically dependent on the glucose oxidase used. In preliminary tests we found no cross-reaction of the enzyme with fructose, even in the presence of fructose concentrations much higher than those occurring in our samples. Furthermore, every step of the method can be monitored in terms of recovery of ^{14}C-glucose so that precise corrections can eventually be made. This is possible since each plasma sample also contains 3-^{3}H-glucose which is initially counted and used as an internal standard through the subsequent steps involved in the method.

Calculations. Rates of endogenous glucose production and uptake were calculated in the steady state by the isotope dilution equation and during nonsteady states by *Steele's* equations in their derivative form (*Steele* 1959; *Cowan and Hetenyi* 1971). The time curves for glucose concentration and specific activity were fitted with polynomial functions by the method of least squares. The value of 0.65 of the initial glucose pool was used as the rapidly mixing compartment to compensate for nonuniform mixing with the entire glucose space (*Cowan and Hetenyi* 1971). The evaluation of the rates of glucose turnover based on the primed-continuous infusion and the pool fraction technique has recently been validated for both steady and nonsteady states (*Radziuk, Norwich and Vranic* 1978). The conversion of the administered fructose to plasma glucose was quantitated by the following parameters: 1) rate of fructose conversion to glucose (mg/kg min^{-1}); 2) per cent of glucose derived from fructose; and 3) per cent of fructose converted to glucose. The first parameter was calculated by dividing the rate of appearance of ^{14}C-glucose by the specific activity of plasma ^{14}C-fructose. The rate of appearance of ^{14}C-glucose was obtained by *Steele's* equation, as described above, using plasma 3-^{3}H-glucose concentration and replacing the concentration of cold glucose with that of ^{14}C-glucose. This method has been validated in previous studies by comparing the rates of ^{14}C-glucose production determined isotopically with those obtained simultaneously with hepatic A-V difference technique (*Chiasson, Liljenquist, Jennings, Lacy and Cherrington* 1977). The per cent of glucose derived from fructose was calculated by dividing the rate of glucose production from fructose (which equals the rate of fructose conversion to glucose) by the rate of hepatic glucose production. Finally, the per cent of fructose converted to glucose was obtained by dividing the amount of glucose derived from fructose by the rate of fructose utilization. The latter is equal to the rate of fructose infusion during steady state conditions, when the plasma concentration of fructose is stable. This condition was actually present for the greater part of the fructose infusion, except in the first 40–60 min. As previously shown by *Chiasson* et al. (1977), by comparing the double isotope method to the hepatic A-V difference technique, the current approach provides an accurate estimate of the rate at which a circulating precursor is converted to glucose. However, it should be noted that this parameter cannot be equated to the rate of glucose production via gluconeogenesis due to the possible exchange of ^{14}C-atoms in the hepatic oxalo-acetate pool. Nevertheless, it certainly provides a reliable index of gluconeogenesis *in vivo*. Statistical analysis was performed with the Student's t-test for paired samples. Data are presented as means ± SE.

Results

Figure 1 shows the changes in plasma fructose and glucose concentration, and in glucose turnover during intravenous fructose infusion. Plasma fructose concentration increased rapidly to about 20 mg/dl by 50–60 min and remained remarkably stable for the rest of the infusion period. Plasma glucose (100 ± 4 mg/dl, pre-infusion) fell by 8–10 mg/dl in the first 40 min of fructose infusion ($p < 0.005$) and remained unchanged thereafter. This mild decrease of plasma glucose concentration was due to a temporary fall in the rate of glucose output (3.07 ± 0.2 mg/kg min^{-1}, pre-infusion) which reached levels 33% below baseline at 10 min ($p < 0.005$) and then returned slowly to basal levels by 40–50 min. Glucose uptake decreased slightly

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RECOVERY MECHANISMS FROM ACUTE HYPOGLYCAEMIA IN COMPLETE TETRAPLEGIA

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Abstract. Acute hypoglycaemia has been achieved in tetraplegic subjects and in healthy controls using insulin. The recovery from hypoglycaemia has been followed by measuring appropriate substrate levels in blood. Abnormal responses of lactate and free fatty acid concentrations were observed; blood glucose recovery proceeded at a normal rate.

Key words: Hypoglycaemia; Tetraplegia; Blood lactate; Free fatty acids.

Introduction

IN the tetraplegic patient, transection of the cervical cord above the first thoracic segment interrupts the major motor and sensory tracts and also disrupts the efferent sympathetic pathway. In effect this produces a total pre-ganglionic sympathectomy. This can result in characteristic clinical problems such as reflex hypertension following bladder distension. Metabolic consequences of this autonomic denervation have received little attention, and could be of practical significance in situations such as prolonged fasting during general anaesthesia. We have therefore examined the metabolic response to acute hypoglycaemia in tetraplegia.

Methods

Six normal male subjects (age range 22–64 years) and four male tetraplegic patients (age range 20–44 years) with complete post-traumatic cervical cord transections at the C5/6 level, were studied after an overnight fast. All subjects gave informed consent to the study and none were greater than 10 per cent over their ideal body weight. After resting supine in bed for at least 30 minutes an intravenous teflon catheter was inserted for blood sampling. Soluble insulin (0·15 units/kg body weight) was given intravenously; blood samples were withdrawn in control subjects at regular intervals for 180 minutes after the onset of the hypoglycaemic reaction. This was manifested as a sudden tachycardia coinciding with the symptoms of hypoglycaemia in the normal group. In the tetraplegic subjects blood samples were taken for 210 minutes after the administration of insulin.

Continuous heart rate was monitored by pulse and ECG tape recording, and skin surface sweating was measured in all of the normal and two of the teiraplegic subjects (Cohen, 1966). Serial estimates of blood glucose (Hill & Kessler, 1961), blood lactate (Hohorst, 1970) and plasma non-esterified fatty acids (NEFA'S) (Baird *et al.*, 1967) were made. Results are expressed as mean vlaues $\pm$ one standard error of the mean (S.E.M.).

Results

In the normal subjects the autonomic hypoglycaemic reaction occurred between 20 and 44 minutes (mean 30 minutes) after the administration of insulin. Pulse rate rose from 60 ± 2 to 89 ± 3 beats per min at the time of the initial hypoglycaemic symptoms. In all the control subjects sweating occurred coincidentally with the tachycardia and the onset of subjective hypoglycaemia. In the tetraplegic group no change in resting pulse (63 ± 5 beats/min) was observed, and in the two subjects in whom skin sweating was monitored, there was no detectable increase with hypoglycaemia. Both groups experienced mild degrees of typical neuroglycopenic symptoms of hunger and drowsiness.

The changes in the metabolic parameters measured in both groups are shown in Figures 1–3. Blood glucose fell rapidly from similar mean basal levels in control and tetraplegic subjects following the administration of insulin with a more profound fall in the normal group (Fig. 1). The rate of recovery of blood glucose was parallel in the two groups and in the tetraplegic subjects the mean basal fasting level was attained by 150 minutes after insulin injection.

In the control group mean blood lactate levels doubled after hypoglycaemia with a subsequent steady decline to normal fasting values (Fig. 2). In the tetraplegic subjects the mean basal lactate level was similar, but the striking increase after hypoglycaemia was not observed.

Following the injection of insulin, non-esterified fatty acids initially fell in the control group, then rose coincidentally with blood glucose recovery, increasing to above basal levels (Fig. 3). In the tetraplegic subjects the mean fasting N.E.F.A. level was higher and a similar fall was noted following insulin. However, the

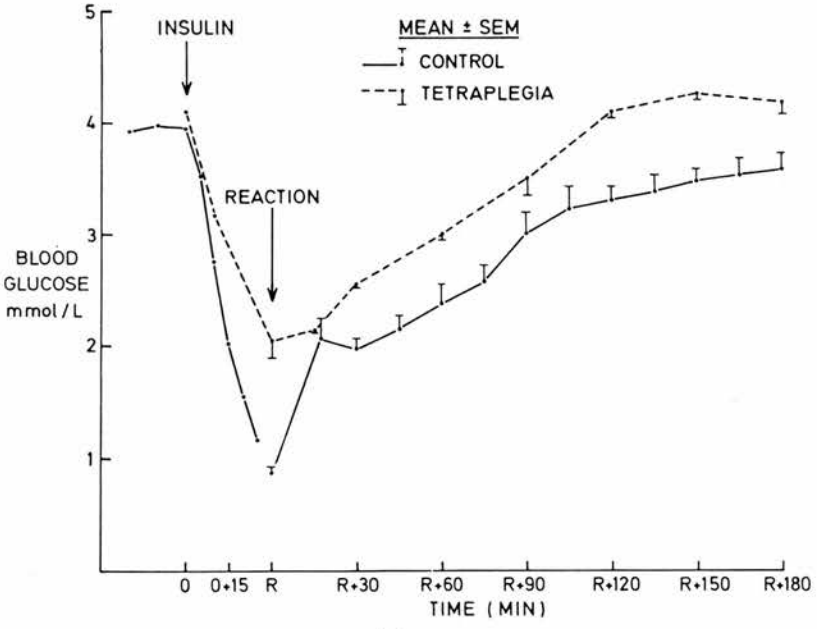

FIG. 1

Changes in blood glucose following injection of insulin in control and tetraplegic subjects.

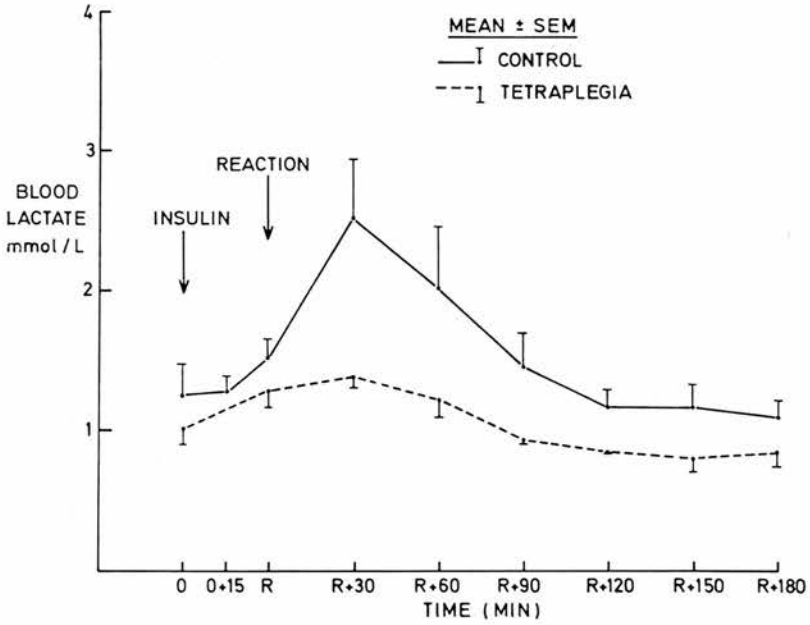

FIG. 2

Changes in blood lactate following injection of insulin in control and tetraplegic subjects.

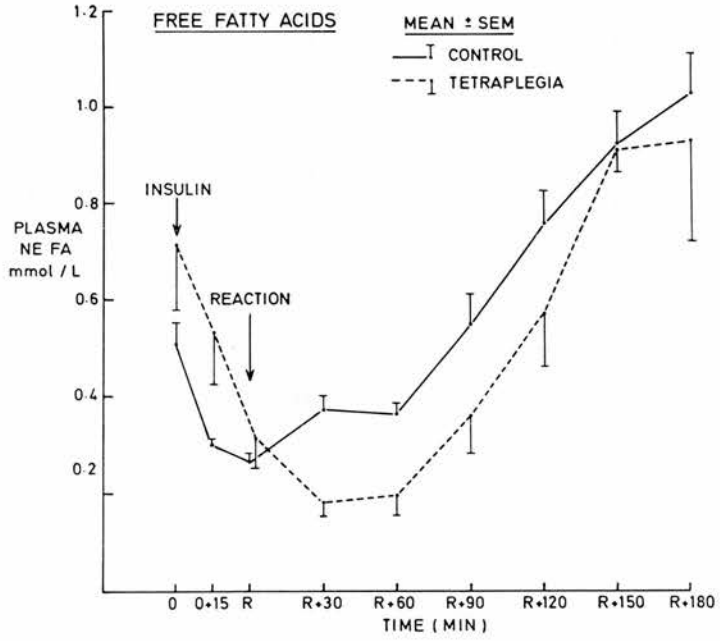

FIG. 3

Changes in plasma free fatty acids following injection of insulin in control and tetraplegic subjects.

subsequent increase in N.E.F.A. levels was delayed compared to the control group.

Discussion

Electrical stimulation experiments in the rat have indicated that the activation of recovery from acute hypoglycaemia originates in the ventromedial nucleus of the hypothalamus (Frohman & Bernardis, 1971). Efferent pathways connect with nerve cell bodies in the thoraco-lumbar grey matter which emerge as the sympathetic outflow and synapse in peripheral ganglia. The adrenal medulla may be considered a modified ganglion, the activation of which causes humoral secretion of adrenaline and noradrenaline (Bloom *et al.*, 1975). The liver with its central role in carbohydrate homeostasis receives a rich sympathetic nerve supply (Holzbauer & Sharman, 1972). Adrenaline and noradrenaline levels rise markedly in peripheral blood following hypoglycaemia and the initiation of recovery from hypoglycaemia bears a close temporal relationship to this acute secretion (Garber *et al.*, 1976). Blood glucose recovery is also impaired by adrenergic blockade (Corrall *et al.*, 1978) and during total autonomic blockade with hexamethonium (Lawrence & Stacey, 1952).

Following the injection of insulin, a lesser fall of blood glucose occurred in the tetraplegic subjects. This relative insulin insensitivity is a recognised consequence of physical inactivity (Lipman *et al.*, 1970). The rate of recovery in the tetraplegic group, however, is similar to that observed in the control subjects, indicating that recovery from hypoglycaemia is relatively normal. This suggests that non-adrenergic mechanisms may be invoked to restore blood glucose to normal. However, it is possible that carbohydrate homeostasis could be severely impaired during the early period following traumatic transection of the cervical cord, and this merits further study.

The lack of response of blood lactate to hypoglycaemia in the tetraplegic group is an interesting finding. The blood lactate rise following hypoglycaemia is thought to result from breakdown of muscle glycogen and this is activated by raised circulating adrenaline levels. Thus it is blocked by hexamethonium which prevents secretion of adrenaline, and also by adrenalectomy (Di Salvo *et al.*, 1956). The formation of glucose from lactate has been implicated as an important factor in the recovery from hypoglycaemia in man (Corrall *et al.*, 1978). It is of interest therefore that recovery proceeds normally in the tetraplegic group where lactate production appears to be impaired.

In both the control and tetraplegic subjects the fall in N.E.F.A. levels following insulin injection results from inhibition of fat cell lipolysis. The subsequent re-activation of lipolysis is delayed in the tetraplegic subjects and probably results from adrenergic denervation. In the later stages of blood glucose recovery, other lipolytic hormones including glucagon, growth hormone and cortisol may be responsible for the raised N.E.F.A. levels which are eventually attained.

These preliminary observations indicate that following cervical cord transection in man significant metabolic dysfunction occurs. Blood lactate and plasma N.E.F.A. responses to hypoglycaemia are abnormal, but restoration of euglycaemia appears to be relatively intact.

Summary

The metabolic response to insulin-induced hypoglycaemia was studied in six normal and four tetraplegic subjects. In the tetraplegic group the normal

autonomic reaction to hypoglycaemia was absent, but blood glucose recovery was not impaired. The normal increase in blood lactate was markedly attenuated and the rise in plasma free fatty acids following hypoglycaemia was delayed. The significance of these metabolic changes in tetraplegia is discussed.

RÉSUMÉ

L'hypoglycémie aigüe a été provoquée chez les sujets tétraplégiques et les cas contrôles sains par l'utilisation de l'insuline. Le rétablissement de l'hypoglycémie a été étudié en mesurant les niveaux substratums appropriés dans le sang. Des réactions anormales de lactate et de concentrations acides gras libre étaient observées. La guérison du glucose du sang continua à un cours normal.

ZUSAMMENFASSUNG

Die akute Hypoglykämie ist in den tetraplegien Patienten und gesunden Kontrollen durch die Benutzung des Insulins untersucht worden. Die Heilung der Hypoglykämie ist in der Blutglukose studiert worden. Abnormale Werte von Lacticaecid und von freien, fettigen Säuren wurden gemessen.

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AUTONOMIC NEURAL CONTROL MECHANISMS OF SUBSTRATE AND HORMONAL RESPONSES TO ACUTE HYPOGLYCAEMIA IN MAN

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SUMMARY

The contributions of adrenergic and cholinergic mechanisms to recovery from acute hypoglycaemia induced by insulin (0·15 units/kg i.v.) were examined in eleven normal subjects, six subjects with a pre-ganglionic sympathectomy (adrenergic denervation) and six sympathectomized subjects given atropine (combined adrenergic denervation and cholinergic blockade).

Blood glucose recovery was impaired only in the sympathectomized subjects given atropine. The blood lactate response was reduced and the rise in free fatty acids was delayed in both groups of sympathectomized subjects, in whom the normal rises of plasma cyclic AMP and noradrenaline were absent. The plasma pancreatic glucagon response was appropriate to the prevailing blood glucose concentrations in all three groups. The cortisol response was impaired and the pattern of ACTH secretion was abnormal in sympathectomized subjects given atropine. Growth hormone levels were higher in both sympathectomized groups.

Blood glucose homeostasis was impaired during combined adrenergic denervation and cholinergic blockade. Glucagon secretion was activated independently of vagal control. In the sympathectomized group given atropine, the rise in plasma cortisol was blunted despite a greater degree of hypoglycaemia. A blockade of central cholinergic receptors producing impaired activation of ACTH secretion at hypothalamic level may explain, at least in part, this delayed restoration of normoglycaemia.

Acute hypoglycaemia is associated with an activation of the sympatho–adrenal system which produces classical autonomic symptoms. Early investigators drew attention to the possible relationship between activation of this system and the initiation of metabolic

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recovery (Cannon *et al.*, 1924; Houssay *et al.*, 1924), and subsequent studies concentrated on adrenergic mechanisms. During acute hypoglycaemia however, there is evidence for the activation of post-ganglionic cholinergic nerves which promote gastric acid secretion and innervate skeletal muscle (Allwood & Ginsburg, 1959) and stimulate the parotid salivary glands (Corrall *et al.*, 1976). Furthermore, a vagal cholinergic mechanism has been implicated in the control of glucagon (Bloom *et al.*, 1974a; 1974b) and pancreatic polypeptide secretion (Adrian *et al.*, 1977) in response to hypoglycaemia. In the present paper we describe studies in man in which we have examined the contributions of adrenergic and cholinergic activity to the homeostatic recovery mechanism from acute hypoglycaemia.

SUBJECTS

Control group: Eleven normal volunteers (nine male, two female), age range 20–29 years, were all within 10% of their ideal body weight, and taking no medications.

Tetraplegic group: Six, non-obese male patients, age range 21–44 years, with complete post-traumatic transection of the cervical spinal cord above C7, which had occurred at least 6 months earlier (Table 1).

Tetraplegic and atropine group: Six, non-obese male tetraplegic patients (two from the above group) with a similar neurological lesion, age range 19–28 years (Table 1).

Informed consent was obtained from all subjects, none of whom had known endocrine or metabolic disorders. The protocol was approved by the local Medical Ethics Committee.

METHODS

All subjects were studied supine after an overnight fast. Insulin (0·15 u/kg body weight) was injected after basal blood samples had been withdrawn through an indwelling teflon

Table 1. Clinical details of tetraplegic subjects (two groups)

Subject (no.)	Age (years)	Duration of cervical transection	Level of transection
With atropine			
1	21	5 years	C4/5
2	22	9 months	C6/7
3	19	1 year	C5/6
4	28	4 years	C5/6
5	27	6 months	C4/5
6	19	4 months	C4/5
Without atropine			
1	21	5 years	C4/5
2	22	8 months	C6/7
7	44	13 years	C5/6
8	23	6 months	C5/6
9	40	19 years	C5/6
10	35	7 years	C5/6

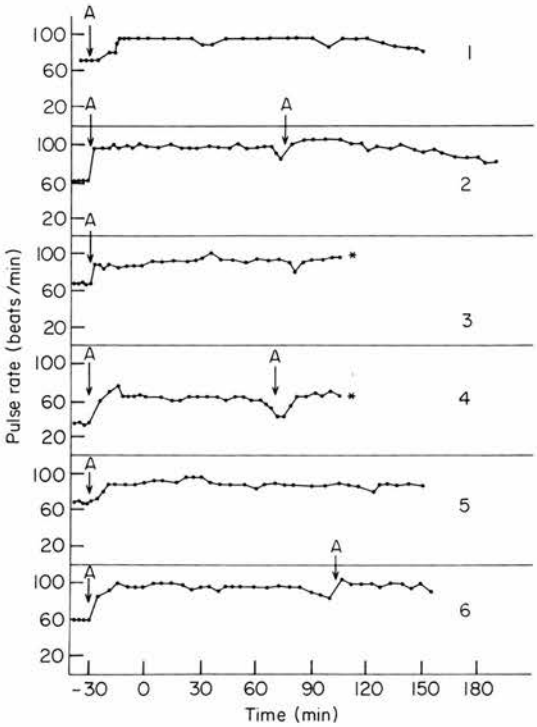

Fig. 1. Pulse rate in individual tetraplegic subjects given atropine. Insulin was administered at time 0 min. A = atropine (i.v.); *discontinued because of neuroglycopenia.

cannula. Atropine (15 μg/kg body weight) was administered 30 min prior to the insulin in six tetraplegic subjects. The heart rate was monitored, and if necessary, a further dose of atropine was administered (Fig. 1).

Serial blood sampling was performed for 210 min after the administration of insulin, and measurements of blood glucose (Hill & Kessler, 1961), blood lactate (Hohorst, 1970), plasma free fatty acids (FFA) (Baird *et al.*, 1967), plasma cyclic AMP (cAMP) (Brown *et al.*, 1972), C-terminal glucagon-like immunoreactivity (Stout *et al.*, 1976), plasma noradrenaline (Henry *et al*, 1975), plasma cortisol (Mattingly, 1962), plasma ACTH (Feek *et al.*, 1981) and plasma growth hormone (GH) (Hunter, 1976) were taken.

Statistics

Results are expressed as mean ± the standard error of the mean (SEM). Statistical significance between groups of subjects was estimated using Student's *t* test for paired (within group) or unpaired data, except when data was not normally distributed about the mean value. The Wilcoxon rank test was used to assess statistical significance for pancreatic glucagon, GH and ACTH which were not distributed in a normal manner.

RESULTS

Approximately 30 min after the injection of insulin the normal subjects experienced a typical hypoglycaemic reaction, manifested by sweating, tachycardia and symptoms of

neuroglycopenia. In the tetraplegic patients without atropine there was no consistent change in pulse rate, sweating was not observed and only mild neuroglycopenia occurred. With atropine, the tetraplegic patients all had an elevated heart rate (Fig. 1), and during hypoglycaemia, visible sweating was absent, but the neuroglycopenic symptoms were worse and more persistent. Two of these patients complained of severe neuroglycopenia during cholinergic blockade, which necessitated discontinuing the study with parenteral glucose 90 min after insulin.

Plasma substrate concentrations (Fig. 2)

Blood glucose. Following insulin, the rate of fall of blood glucose was slower in both groups of tetraplegic subjects and was significantly different from the normal group at 30 min ($P < 0{\cdot}001$). In the group of tetraplegic patients who did not receive atropine, blood glucose recovery proceeded at a normal rate. With atropine, blood glucose recovery in the tetraplegic patients was greatly impaired, in comparison with both the control group and

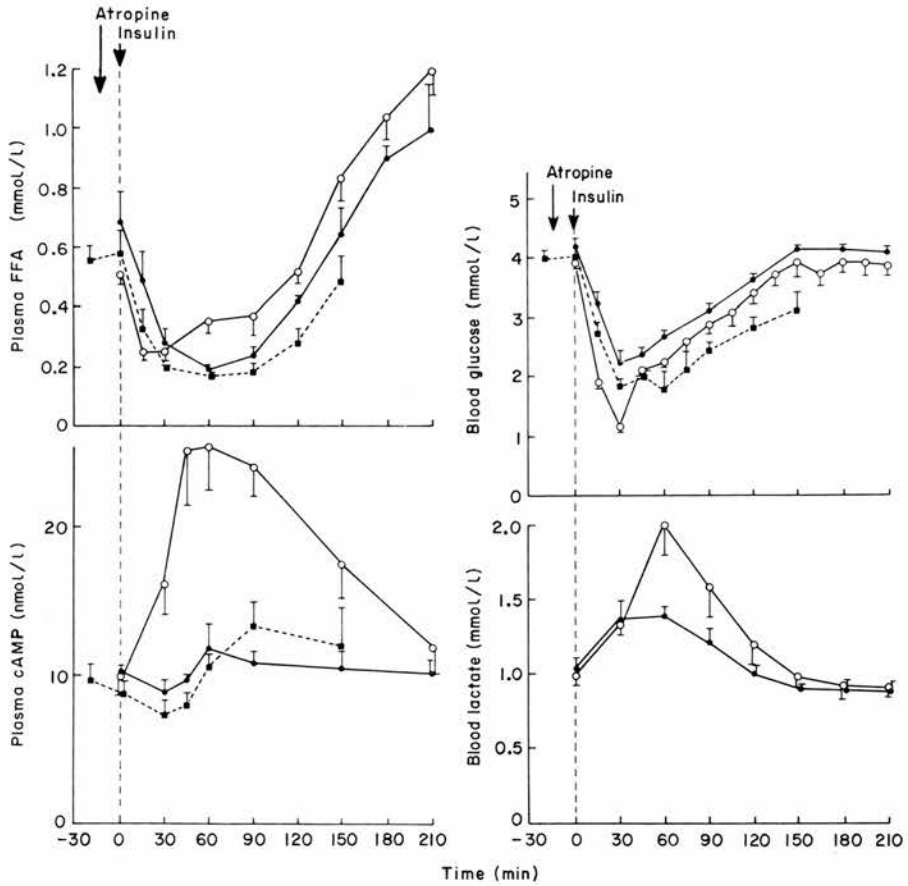

Fig. 2. Changes in blood glucose, blood lactate, plasma free fatty acids and plasma cyclic AMP concentrations (mean ±SEM) in response to insulin-induced hypoglycaemia, in normal subjects and in tetraplegic subjects with or without atropine. Insulin was administered at time 0 min. Mean ±SEM, ♀ normal; ♿ tetraplegia; ♿ tetraplegia and atropine.

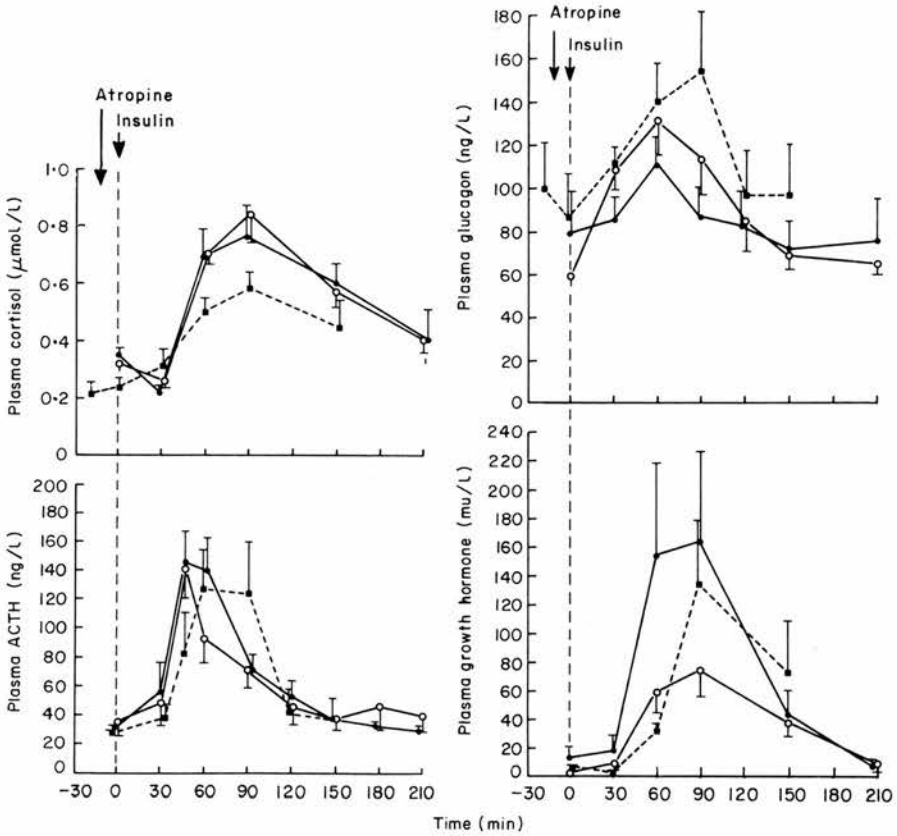

Fig. 3. Changes in plasma pancreatic glucagon, growth hormone, cortisol and ACTH (mean ±SEM) in response to hypoglycaemia in normal subjects and in tetraplegic subjects with or without atropine. Insulin was injected at time 0 min. Mean ±SEM, ♀ normal; ♀ tetraplegia; ♀ tetraplegia and atrophine.

the tetraplegic group without atropine, at 60 min ($P < 0{\cdot}01$), 90 min ($P < 0{\cdot}001$) and 150 min ($P < 0{\cdot}001$).

Blood lactate. In the control subjects blood lactate increased to a peak value at 60 min ($P < 0{\cdot}001$ v. mean basal value of control group) (Fig. 2). In the tetraplegic subjects without atropine the mean blood lactate at 60 min was significantly lower ($P < 0{\cdot}05$) than the corresponding value observed in the control group. Blood lactate was measured in two tetraplegic subjects given atropine; these were similar to the levels observed in the tetraplegic group without atropine.

Plasma FFA. In all three groups, mean plasma FFA levels fell after insulin (Fig. 2). A subsequent rise was observed in the control group to levels above basal and a similar, but delayed, pattern was present in the tetraplegic groups. This delay was more pronounced in the group given atropine.

Plasma cAMP. In the control group, mean plasma cAMP increased rapidly to a peak more than double the basal value at 30 min. This rise was absent in both groups of tetraplegic patients.

Plasma hormone concentrations (Fig. 3)

Plasma pancreatic glucagon. Mean plasma pancreatic glucagon rose to a peak value at 60 min in the control group and in the tetraplegic group without atropine. In the tetraplegic group with atropine the pancreatic glucagon secretory response was prolonged, reaching a peak value at 90 min, which was not significantly greater than the control group ($P < 0{\cdot}1$) but was greater than the tetraplegic group without atropine ($P < 0{\cdot}02$).

Plasma noradrenaline. In the control group the plasma noradrenaline rose from a mean basal value of 201 ± 103 ng/l to 430 ± 114 ng/l at 45 min after insulin. In both tetraplegic groups, the mean basal values were lower than the control group at 25 ± 6 ng/l (without atropine) and 63 ± 24 ng/l (with atropine), with no measurable response to hypoglycaemia.

Plasma cortisol. The rise in plasma cortisol (Fig. 3) was very similar in the control subjects and in the tetraplegic subjects without atropine. In the tetraplegic group given atropine, despite prolonged hypoglycaemia, plasma cortisol was lower. This difference was significant at 90 min compared with the control group ($P < 0{\cdot}05$).

Plasma ACTH. In the control group, and in the tetraplegic group without atropine, plasma ACTH levels rose to a peak value at 45 min. A different pattern of secretion was observed in the tetraplegic group given atropine, with a slower rise to a peak at 60 min, and a higher mean value than both of these groups at 90 min, but this did not achieve statistical significance.

Plasma GH. In the control group the expected rise in plasma GH levels was observed following hypoglycaemia, but in both tetraplegic groups the peak mean values of GH at 60 min were more than double that of the control group. This was not statistically significant.

DISCUSSION

The production of a pre-ganglionic sympathectomy in traumatic tetraplegia provides an excellent model for the evaluation of adrenergic mechanisms *in vivo*. The administration of atropine to these subjects has enabled an examination of residual cholinergic control mechanisms. A significant degree of cholinergic blockade is demonstrated by the persistent elevation in heart rate in the tetraplegic subjects given atropine (Fig. 1).

In the sympathectomized subjects the blood glucose recovery proceeded at a normal rate and was unaffected by the absence of a catecholamine response. In the present study the administration of atropine to sympathectomized subjects significantly impaired blood glucose recovery.

In normal subjects the rise in blood lactate in response to hypoglycaemia results from muscle glycogenolysis (Haugaard *et al.*, 1976). The rises of lactate and FFA following hypoglycaemia are reduced by adrenergic antagonists (Billington *et al.*, 1954; Di Salvo *et al.*, 1956; Werk *et al.*, 1961; Abramson *et al.*, 1966; Abramson & Arky, 1968) and are absent in response to intracellular glucopenia induced by 2-deoxy-glucose in sympathectomized patients (Brodows *et al.*, 1973; 1975). In the present study, the impaired rise following hypoglycaemia in blood lactate and plasma FFA in the sympathectomized subjects can therefore be attributed to adrenergic denervation. Although lactate is an important precursor for hepatic gluconeogenesis during recovery from hypoglycaemia (Young & Landsberg, 1977; Exton, 1979), impairment of lactate production had no effect

on blood glucose recovery in the tetraplegic subjects without atropine, and is unlikely to be the primary cause of the retarded glucose recovery during cholinergic blockade. In the present study the absence of a cyclic AMP rise in response to hypoglycaemia in all tetraplegic subjects, despite normal glucagon secretion, suggests that the activation of hepatic adenyl cyclase by hypoglycaemia is mediated by catecholamines.

The rise in plasma pancreatic glucagon concentrations was proportional to the degree of hypoglycaemia attained in both groups of tetraplegic subjects, confirming that adrenergic mechanisms are not the prime mediators of pancreatic glucagon release following hypoglycaemia in man (Walter *et al.*, 1974; Ensinck *et al.*, 1976; Palmer *et al.*, 1976). Studies of hypoglycaemia in the calf (Bloom *et al.*, 1974a) and in vagotomized human subjects (Bloom *et al.*, 1974b) have suggested that glucagon secretion is primarily via cholinergic vagal control. However, recent human studies have shown that neither truncal vagotomy nor cholinergic blockade prevent glucagon secretion in response to hypoglycaemia (Palmer *et al.*, 1979), and it is apparent from the present studies that secretion can occur independently of these neural control mechanisms.

The delayed rise in plasma ACTH, and the reduced plasma cortisol response in the tetraplegic patients under cholinergic blockade, may have been responsible for the impaired blood glucose recovery in these subjects, despite apparently adequate secretion of pancreatic glucagon and growth hormone. Cortisol influences gluconeogenesis by a permissive effect on the action of glucagon and catecholamines on hepatic glycogenolysis and gluconeogenesis (Exton *et al.*, 1972) and by its effect upon catecholamine-induced release of lactate from skeletal muscle and glycerol from adipose tissue (Steele, 1975). Impaired blood glucose recovery from hypoglycaemia has been described in states of primary and secondary adrenal insufficiency (Fraser *et al.*, 1941; De Bodo & Altszuler, 1958; Shahmanesh *et al.*, 1980), although this was not apparent when replacement corticosteroid therapy had been given prior to the induction of hypoglycaemia (Ginsburg & Paton, 1956; Ensinck *et al.*, 1976, Brodows *et al.*, 1976). A relative deficiency of cortisol following hypoglycaemia might therefore diminish blood glucose recovery through its permissive interaction with glucagon, particularly if the availability of lactate for gluconeogenesis was limited. A cholinergic mechanism has been implicated in the secretion of corticotrophin-releasing factor in the murine hypothalamus (Jones *et al.*, 1976). The present data suggest that a similar mechanism may exist in man; atropine crosses the blood–brain barrier (Innes & Nickerson, 1975) and could have delayed the activation of ACTH secretion in the tetraplegic group under cholinergic blockade.

Recovery from hypoglycaemia requires an integrated activation of various counter-regulatory mechanisms which provide substrates for gluconeogenesis and stimulate hepatic glycogenolysis and gluconeogenesis. The present studies indicate that in the absence of peripheral adrenergic mechanisms alone this homeostatic process is relatively intact. With the administration of atropine both classical components of the autonomic nervous system are impeded and an impaired blood glucose recovery becomes manifest.

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Attenuation of the Pancreatic Beta Cell Response to a Meal Following Hypoglycaemia in Man

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Summary. The plasma concentration of C-peptide, insulin (IRI) and glucose was measured in 9 healthy subjects during insulin-induced hypoglycaemia followed by a meal. Identical observations were made in the same subjects after an equivalent period of fasting without hypoglycaemia (control study). Endogenous secretion of insulin was suppressed following administration of exogenous insulin and this persisted long after the blood glucose concentration had returned to normal. After the meal the mean blood glucose rose to a peak of 8.4 ± 0.3 mmol/l (mean ± SEM) at 60 min and was still raised at 7.5 ± 0.3 mmol/l at 120 min, compared with a peak value of only 5.1 ± 0.2 mmol/l at 30 min after the meal in the control study. Following hypoglycaemia the mean plasma IRI rose from 8.3 ± 1.3 mU/l to a delayed peak of 81.6 ± 12.7 mU/l at 60 min and was 123.5 ± 14 mU/l at 120 min post-prandially, compared with a peak of 72.4 ± 0.5 mU/l at 30 min after the meal in the control study. Acute hypoglycaemia may thus induce an abnormal pattern of insulin secretion in response to a meal, with impaired carbohydrate tolerance in normal subjects.

Key words: Hypoglycaemia, beta-cell function, C-peptide, insulin secretion.

The study of insulin secretion during hypoglycaemia in man has, until recently, been restricted by an inability to interpret levels of immunoreactive insulin (IRI) in plasma following the administration of exogenous insulin [1]. Attempts to circumvent this problem have included the induction of hypoglycaemia by the administration of alcohol [2] or fish insulin [1]. Connecting (C-) peptide and insulin are released in equimolar amounts by the pancreatic beta cell. The development of a radioimmunoassay for connecting peptide reactivity (CPR) has made it possible to study the secretion of insulin in vivo during insulin-induced hypoglycaemia [3–6]. We have extended these observations by examining the response of the beta cell to a meal following acute hypoglycaemia.

Subjects and Methods

Eleven healthy subjects (9 male, 2 female), age range 20–29 years (mean 23.8 years) were studied after an overnight fast. None of the subjects were taking any medications, and all were within ten per cent of their ideal body weight (mean 96 per cent, range 91–102 per cent). The approval of the Medical Ethics Committee was obtained for the study and informed consent was given by each subject.

Hypoglycaemia Study

Crystalline beef insulin (0.15 units/kg. body weight) was administered as a bolus by rapid intravenous injection and blood samples were taken via an indwelling teflon cannula for estimation of blood glucose [7], plasma CPR [8] (effective detection limit 0.06 nmol/l) and plasma IRI levels [9] in the fasting state, and at intervals for 210 min after the injection of insulin. Nine of these subjects were then given a standard mixed meal containing 30g protein, 85g carbohydrate and 40g fat, and blood sampling was continued for a further 120 min. All subjects experienced symptoms and signs of hypoglycaemia between 20 and 30 min (mean 24 min) after the injection of insulin.

Control Study

The same 9 subjects were restudied after an interval of at least one week. The meal was given after an overnight fast plus an equivalent period of fasting without the administration of insulin, and the same parameters were measured. In both studies all subjects consumed the meal within 15 min.

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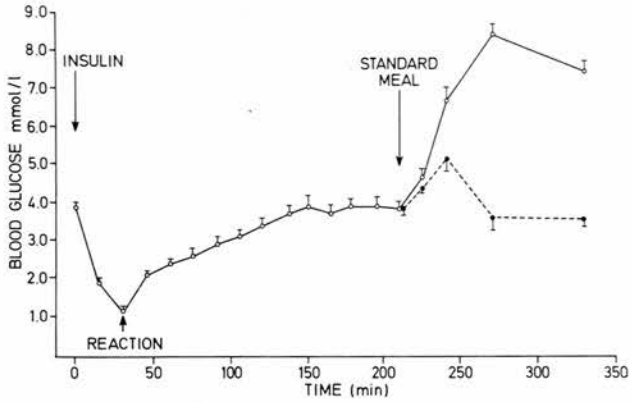

Fig. 1. Blood glucose concentration (mean ± SEM) following injection of insulin and in response to a subsequent meal (Hypoglycaemia study) and in response to a meal alone after an equivalent period of fasting (Control study). ♁—♁ Hypoglycaemia, ♀----♀ Control

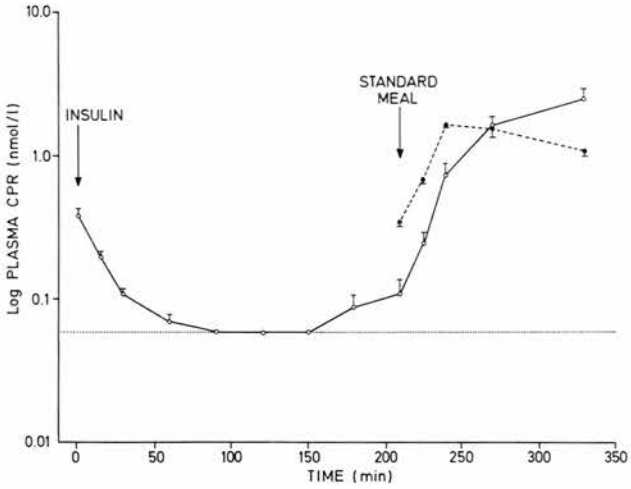

Fig. 2. Log plasma CPR concentration (mean ± SEM) in the hypoglycaemia and control studies. The effective detection limit of the assay is marked as a horizontal dotted line (0.06 nmol/l). ♁—♁ Hypoglycaemia, ♀----♀ Control

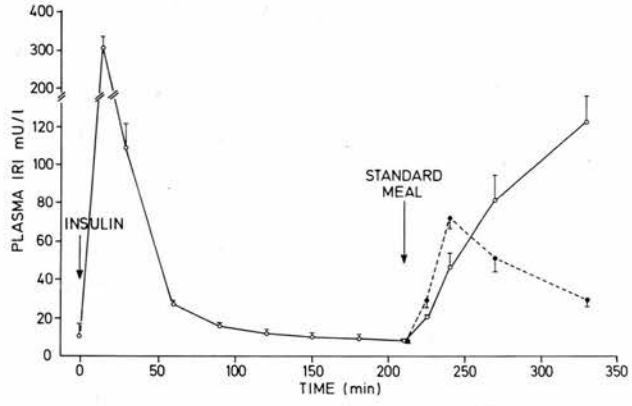

Fig. 3. Plasma IRI concentration (mean ± SEM) in the hypoglycaemia and control studies. ♁—♁ Hypoglycaemia, ♀----♀ Control

The results are expressed as mean ± one standard error of the mean (SEM) and statistical significance was estimated using Student's 't' test.

Results

Blood Glucose

In the hypoglycaemia study the mean fasting blood glucose fell from 3.9 ± 0.1 to 1.2 ± 0.1 mmol/l at the time of the acute hypoglycaemic reaction, and regained the fasting level by 150 min after the injection of insulin (Fig. 1). Following the meal, the mean blood glucose concentration rose to a peak of 8.4 ± 0.3 mmol/l at 60 min and was still raised (7.5 ± 0.3 mmol/l) at 120 min post-prandially.

In the control study the mean blood glucose concentration of the same subjects reached a peak value of only 5.1 ± 0.2 mmol/l at 30 min after the meal and regained the fasting level (3.6 ± 0.3 mmol/l) by 60 min post-prandially. The difference in the mean blood glucose concentrations following the meal between the two studies was highly significant at 30, 60 and 120 min ($p < 0.001$).

Plasma C-peptide

In the hypoglycaemia study the mean plasma CPR fell rapidly after administration of insulin from a fasting level of 0.39 ± 0.04 nmol/l (range 0.20 to 0.69 nmol/l) to the effective detection limit of the assay, 60 min after the injection of insulin (Fig. 2). It remained low throughout the period of blood glucose recovery and at 210 min after insulin was only 0.11 ± 0.03 nmol/l. CPR levels rose following the meal reaching 1.72 ± 0.24 nmol/l at 60 min and 2.58 ± 0.47 nmol/l at 120 min post-prandially.

In the control study, mean plasma CPR rose from 0.35± 0.01 nmol/l to a peak of 1.76 ± 0.08 nmol/l at 30 min, falling to 1.16 ± 0.14 nmol/l at 120 min. The differences in the mean plasma CPR concentrations between the two studies were significant ($p < 0.001$) at 15, 30 and 120 min after the meal.

Plasma Insulin

In the hypoglycaemia study the expected rise in plasma IRI after the injection of exogenous insulin was observed (Fig. 3), and was followed by an expo-

nential fall. At the time of the meal the plasma IRI had returned to the fasting level. After ingestion of food, mean plasma IRI rose from 8.3 ± 1.3 mU/l to 81.6 ± 12.7 mU/l at 60 min and 123.5 ± 14 mU/l at 120 min.

In the control study, the mean plasma IRI rose from 9.1 ± 0.6 mU/l to 72.4 ± 0.5 mU/l at 30 min, falling to 51.0 ± 7.2 mU/l at 60 min and 29.5 ± 4.5 mU/l at 120 min. The differences in plasma IRI between the two studies were statistically significant ($p < 0.001$) at all four times of measurement after the meal.

Discussion

The value of the CPR assay as an index of endogenous insulin secretion is now well recognised [3–6]. The plasma IRI concentrations after the administration of endogenous insulin closely resemble those reported by Garber et al. [10] and had fallen to within the fasting range prior to ingestion of the meal. The decline in the mean plasma IRI concentration during recovery from hypoglycaemia was not however comparable with the marked fall in plasma CPR concentration to undetectable levels, and is inconsistent with the short half-life of insulin in plasma. Appropriately low levels of plasma IRI were observed in three subjects with concentrations falling to less than 6.0 mU/l by 150 minutes after the administration of insulin, but we are unable to explain the overall discrepancy between mean plasma IRI and CPR concentrations prior to the meal. The prolonged suppression of plasma CPR concentration following hypoglycaemia is consistent however with the observations of Horwitz et al. [3].

The demonstration in man of impaired tolerance to oral glucose following the administration of insulin has previously been described [11] and attributed to the presence of insulin antagonists. The response of the pancreatic beta cell to the ingestion of food following hypoglycaemia has not been reported. The present study shows that following recovery from hypoglycaemia, insulin secretion in response to a meal is abnormal and is associated with impaired carbohydrate tolerance. The initial delay in the secretion of insulin after hypoglycaemia must be partly responsible for the elevated post-prandial blood glucose concentrations. The hypersecretion of insulin observed 120 minutes after the meal may be a direct response to sustained hyperglycaemia.

The mechanism underlying the abnormal secretion of insulin in response to a meal after hypoglycaemia has not been elucidated. The possibility that insulin directly inhibits its own secretion has been studied by maintaining euglycaemia with a glucose infusion during the administration of insulin [12–14]. Partial suppression of CPR levels without hypoglycaemia was interpreted as evidence for the existence of such a direct negative feedback [12, 13] but this was not confirmed by Shima et al. [14]. Plasma catecholamines, which inhibit insulin secretion and rise markedly during hypoglycaemia [10], could also cause pancreatic beta cell suppression. The pattern of insulin secretion in the present study is similar to that observed during and after the infusion of adrenaline, where insulin secretion is suppressed until adrenaline is discontinued [15, 16]; insulin secretion is initially delayed during the recovery period but subsequently hypersecretion of insulin is observed. The early inhibition of insulin secretion after food ingestion could be explained by altered function of the enteroinsular axis following hypoglycaemia. Plasma gastroinhibitory peptide and pancreatic polypeptide levels rise during hypoglycaemia [13, 17, 18], but the response of those hormones to the subsequent ingestion of food is not known. Finally, the secretion of insulin could be inhibited by a direct effect of hypoglycaemia on the metabolism of the beta cell.

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